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JOANNE WILLCOCK

Mycoflora of wheat straw: effects of environmental factors on spoilage and straw quality

Supervisor: Cranfield University, Dr. N. Magan

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ABSTRACT

The effect on the mycoflora, succession and deterioration of straw quality over a range of environmental conditions, including 0.75 to 0.98 water activity (a_w), 10-30^{0}C and pH 4.4-6.4 were investigated. Isolation and colonisation of natural mycoflora and individual fungal species were significantly influenced by temperature x a_w x pH interactions (P <0.05). Generally, the least growth occurring at 0.75 a_w and 10^{0}C, and the maximum at 0.98 a_w and 30^{0}C. Small scale respiratory apparatus was modified to measure respiration of the natural mycoflora and five individual species (*Alternaria alternata*, *Cladosporium cladosporioides*, *Eurotium amstelodami*, *Fusarium culmorum* and *Penicillium aurantiogriseum*) on wheat straw for the first time. Respiratory activity significantly increased with increasing temperature and a_w (P <0.05). At the highest temperature (30^{0}C) there was almost a linear increase, whereas at the lowest temperature (10^{0}C), a lag time occurred prior to increased respiratory activity. Maximum dry matter losses (DML) were found to be 3.40 % at 30^{0}C and 0.98 a_w. Dry matter losses were found to change with storage period, and temperature x a_w interactions. Significant loss of the carbohydrate components (P <0.05), assuming no loss of true lignin, was observed at 20^{0}C when the apparent lignin content rose with increasing a_w and, once 0.90 a_w had been reached, remained at 140 % of the starting value. Of three biocides (Adesol 20, Busan 881 and Lastil 40), Lastil 40 was found to be most effective in vitro and on straw of significantly reducing fungal populations. Field trials with biocides and Nutri-Shield® at different storage moisture contents demonstrated that wads of straw on the surface of the top bales of the stack at 15 and 23 % moisture content provided the most effective storage treatment and significantly conserved the straw quality (P <0.05). All of the treatments, except wads of straw in nets, had increased apparent lignin contents, which showed that degradation had occurred.

To my husband Clive

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LITERATURE REVIEW

1.1 WHEAT STRAW COMPOSITION

Wheat straw consists of leaf sheaths, stems, nodes and senesced leaves after the crop has been harvested. The major components of straw are cellulose and hemicellulose which contribute 75-80% of the dry weight (Harper and Lynch, 1985), the remaining constituents are lignin (14%) and water soluble components that make up 10% of the straw weight and include 5% of the total carbon (Harper and Lynch, 1981).

Straw from different crops has a similar composition to that of wheat straw, unless it is not fully mature, when the proportions of water soluble materials are greater. In straw, about 50% wet weight of the cellulosic fraction does not appear to be bound to the lignin and its degradation proceeds fairly rapidly following the priming action of the water-soluble components. The remaining cellulosic fraction appears to be bound to the lignin and is less available, although this fraction is generally degraded before the lignin.

Cellulose is a linear polymer made of glucose subunits linked by β-1,4 glucosidic bonds. Each glucose residue is related by 180^0 relative to its neighbour and the basic repeating unit is called cellobiose. The chain lengths vary between 100 and 14,000 residues. These cellulose chains form numerous intra- and inter-molecular hydrogen bonds, which account for the formation of rigid, insoluble microfibrils.

Microfibrils are orientated in parallel and form highly ordered crystalline domains interspersed by more disordered amorphous regions (Beguin and Aubert, 1994). The role of cellulose is exclusively structural and the high tensile strength enables plant cells to withstand osmotic pressure and mechanical stress.

Hemicelluloses, like cellulose, are polymers of anhydro-sugar units linked by glycosidic bonds. Unlike cellulose, it is composed of more than one kind of sugar unit, with xylan and glucomannans as the main sugars. Hemicellulose molecules are much shorter and branched and as a result are usually non-crystalline. Lignin is a highly branched, random polymer generated by the free-radical condensation of aromatic alcohols (Gold *et al.*, 1989). Lignin is less easily degraded than hemicellulose or cellulose by micro-organisms.

1.2 MYCOFLORA OF STRAW PRE AND POST-HARVEST

The mycoflora of wheat straw changes continuously. Microbial colonisation initially starts as soon as the cereal plant emerges and continues during growth, senescence, harvesting and storage. In temperate climates fungi colonising straw before harvest are referred to as field fungi and those growing after harvest as storage fungi (Christensen and Kaufmann, 1969). Some fungi have also been classified as intermediates between field and storage groups, for example *Fusarium* species (Pelhate, 1968) which can initiate spoilage at intermediate moisture contents.

The most common field fungi include species of *Cladosporium*, *Alternaria*, *Epicoccum*, *Verticillium*, pink and white yeasts and *Aureobasidium* (Lacey, 1980a). Harvested straw is often colonised by low levels of storage fungi depending on the weather conditions at harvest (Magan, 1988a). Under conducive environmental conditions the storage fungi can initiate spoilage.

About one hundred species have been isolated from wheat straw in previous research. Fungal populations slightly vary depending on whether the crop is a freshly harvested standing crop, chopped or baled. Research by Magan (1988a) found that the standing crop had a fungal population of $2.77x10^7$ colony forming units ($CFUg^{-1}$), with the bacterial population larger by a factor of 10, whereas baled straw had a population of $1.69x10^6$ $CFUg^{-1}$. Mouldy straw may contain up to 10^9 actinomycetes and 10^8 fungal $CFUg^{-1}$. When disturbed these substances can give rise to 10^9 to 10^{10} spores m^{-3} air (Lacey, 1968). Research by Kotimaa (1990) found that 3.7×10^6 $CFUm^{-3}$ air were released from straw samples in Finland.

In wet straw, field fungi often dominate causing predominant spoilage. The dominant storage fungi include *Aspergillus, Penicillium* and *Eurotium* species. These micro-organisms are able to initially utilise the water-soluble components of straw enabling the production of cellulases which can initiate cellulose breakdown and considerably alter the fibre structure. Thus this change in lignocellulosic structure will affect fibre quality and limit its industrial uses.

After storage, straw samples could be classified microbiologically as good (up to 5×10^6 actinomycetes and with fungal populations of 10^6 $CFUg^{-1}$ dry weight), mouldy (up to 2×10^7 actinomycetes and with fungal populations of 2×10^7 CFU g^{-1} dry weight) and very mouldy (up to 8×10^8 actinomycetes and 9×10^7 fungal $CFUg^{-1}$ dry weight) (Lacey, 1968) . The occurrence of different types of fungi during storage are dependent on the environmental conditions; particularly water availability and temperature. At lower water availability the fungi include *Eurotium repens*, *E.amstelodami*, *Aspergillus versicolor* and *A.candidus* (Lacey, 1980a).

With higher water availability and heating large numbers of the species *Rhizomucor pusillus*, *Absidia ramosa* and *Aspergillus fumigatus* are present after several days of storage. *A.fumigatus* is able to grow over an extremely wide temperature range from 15^0C to 55^0C. These fungi die and are replaced by *Humicola lanuginosa*, *Chaetomium thermophile* and *Penicillium dupontii* during the maximum heating phase. During the cooling phase *A.fumigatus* reappears, joined by *Fusarium culmorum*, *Sporotrichum thermophilum* and *Coprinus* sp. (Chang and Hudson, 1967). *Chaetomium* spp. will grow abundantly in damp straw in the absence of heating (Chang and Hudson, 1967). Under damp conditions, the microflora develops in a similar way to that in hay (Pelhate and Agosin, 1985). Many of the fungi isolated are able to utilise cellulose, hemicellulose and a range of simple sugars.

Previous research on fungal colonisation of straw has predominantly concentrated on decomposition after incorporation into the soil environment (Harper and Lynch, 1981 ; Harper and Lynch, 1985 ; Robinson *et al.*, 1993; 1994) or other crops such as barley grain, (Flannigan, 1978; Clarke and Hill, 1981) wheat compost (Chang and Hudson, 1967) or bagasse (Sandhu and Sidhu, 1980).

Research by Magan and Lynch (1986) investigated the cellulolytic activity of 10 soil fungi that colonise cereal crop residues. They found that *F.culmorum* and *Trichoderma harzianum,* common fungal contaminants of straw, colonised straw pieces best at high water availability (-0.7 MPa). *Trichoderma* spp. degraded cellulose, the degradation decreasing with water availability in the range -0.7 to -2.8 MPa at 20^0C. Work by Lacey (1980a) and Magan (1988a) considered fungal colonisation and decomposition of straw in the absence of soil, but possible

differences in fungal populations from different cultivars still needs to be examined.

1.3 PROBLEMS ARISING FROM STORAGE CONDITIONS

Agricultural products and residues, in particular cereals and oil seeds, are normally held under storage for variable periods of time. During storage growth of fungi on straw can result in its degradation and loss of dry matter. Mould growth in harvested grain can cause a decrease in germinability, a change in nutrient content and quality, the possible production of mycotoxins, and support the growth of a mycoflora that can become a health hazard to man and animals (Tuite and Foster, 1979).

Similar effects are experienced for cereal residues. The reason that fungi cause so much damage relative to other micro-organisms in stored materials is their ability to grow at low moisture levels, much below the level at which bacteria can multiply (Pitt, 1975).

Proper long term storage has become both a microbiological and engineering problem. In most cases a drying regime will reduce the moisture level below that value suitable for mould spore germination on cereal grain. Following this, the material needs to be placed under storage conditions where environmental control by engineering practice will maintain the moisture level at the correct value. Cereal residues, however, are considered to be a waste product, and do not undergo this treatment as it is not economically viable. Therefore extensive knowledge of all the factors that affect fungal growth on cereal residues has not

been investigated thoroughly. Only by producing high value products from these waste materials will this problem be rectified. Losses due to post-harvest diseases are comparatively easy to estimate but data are limited (Harvey, 1978). Losses due to poor storage conditions of cereal residues are more difficult to estimate and little information is known about the extent of losses at on-farm sites. Cereal residues, for example cereal straws, are stored in large high density bales. The difficulty that arises from this, is that the extent of microbial growth and losses incurred from it, within the bales are not easily observed from the exterior. Also within large volumes of stored material changing environmental conditions allows the entry of water and can lead to migration of moisture, condensation and the formation of moist pockets.

Under such conditions fungal spore germination, growth and proliferation can occur. An additional problem demonstrated by Lacey (1974,1980b) was that very damp hay can spontaneously heat resulting in the establishment of thermophilic actinomycetes. The extent of loss of dry matter of cereal residues depends mainly on two important environmental conditions, moisture content and temperature, during storage (Magan, 1988a). There have been few studies on straw but more extensive research into dry matter losses of hay in relation to moisture content is available. Rees (1982) found that during haymaking up to 30% of the dry matter present initially can be lost. Kuntzel *et al.* (1976) added anhydrous ammonia to moist hay and this treatment reduced losses in the stored hay.

Research into the extent of dry matter losses of wheat straw and other cereal straws has mainly concentrated on the decomposition when incorporated into a soil environment and composting. Magan *et al.* (1989) investigated the effect

various water contents had on decomposition of straw in soil. They found that after six months 50% dry matter loss had occurred in wet soil, whereas only about a half of this had occurred in dry soil. Broder and Wagner (1988) studied the decomposition of corn, wheat and soybean residuals in soil and determined that soybean had the most rapid rate of decomposition, followed by corn then wheat. This suggests that each material has its own rate of decomposition.

Research by Chang (1967) investigated losses in mass from wheat straw compost. He found that straw had lost over half of its dry weight after 60 days and the greatest rate of loss occurred over the first 5 days. The loss of total dry weight could be almost completely accounted for by the loss in hemicellulose and cellulose.

Laboratory scale experiments have shown that over a three month period, losses in dry matter in wheat straw stored without soil, above 25% moisture content can reach 15-25% (Magan, 1988a). Sain and Broadbent (1975) found that up to 10% dry matter was lost from rice straw at 16-17% moisture content when stored at between 10-25^{0}C. However, little research has been performed on dry matter losses of wheat straw under various storage conditions, and as yet there has been no research under large scale and on-farm conditions.

1.4 MOISTURE CONTENT AND WATER ACTIVITY

The rate of colonisation of straw by fungi is determined by the prevailing environmental conditions, particularly water availability and temperature. The simplest measure of water in straw is moisture content (Christensen and

Kaufmann, 1969) which is expressed as a percentage of the wet or dry weight of the straw. Although moisture content describes the amount of the water in a material it does not indicate its availability to micro-organisms. Instead equilibrium relative humidity (ERH), water activity (a_w) and water potential provide better measures of the availability of water to the micro-organism (Griffin, 1982).

The water activity of a substance is defined as the ratio of the vapour pressure of water over a substrate to that over pure water at the same temperature and pressure (Lacey, 1980b). Numerically a_w is the same as ERH, but is expressed as a proportion of one rather than as a percentage. It provides a measure of the availability of water in a substrate to organisms growing on it. Conventionally water activity in straw is expressed on a scale of 0-1, where 1 is pure water. The relationship between a_w and moisture content can be expressed by a moisture sorption isotherm, which has a sigmoid curve relationship. For each agricultural substrate the moisture sorption isotherm will vary. Varieties of the same crop can also slightly differ in their moisture sorption characteristics (Pixton and Warburton, 1977). The relationship between a_w and moisture content will also change with temperature (Ken-Youn Li and Torres, 1993).

Moisture sorption isotherm curves are subject to the hysteresis effect. Hills *et al.* (1996) have recently provided an explanation for this phenomenon. During the desorption of an initially water saturated porous material forces dictate that air first penetrates larger pores before smaller pores. Conversely during an adsorption experiment frictional forces in the throats and necks dictate that water first enters the large pores before smaller pores or throats. Implying that at intermediate water

contents there are more water filled large pores in adsorption than in desorption, therefore it would be expected that the adsorption isotherm would give higher a_w than desorption at the same water content. This difference is considered as the hysteresis effect.

There has been extensive research into the effect a_w has on a variety of cereal products; on rice straw (Sain and Broadbent, 1975), on wheat (Duggal and Muir, 1981; Myrold *et al.*,1981; Magan and Lacey, 1984a; Magan and Lynch, 1986; Magan, 1988a;) and on other cereal residues (Bartholomew and Norman, 1944; Snow *et al.*, 1944; Magan and Lacey, 1984b).

Some studies have also examined the effect of a_w x temperature interactions on decomposition of materials by fungi (Ayerst, 1964; Duggal and Muir, 1981; Smith and Hill, 1982; Magan and Lacey, 1984b; Magan, 1988a,b). However, there has been little research into the effect of both factors on stored wheat straw in the absence of soil. More detailed and accurate knowledge is needed in this area, especially on the potential rates of losses and decomposition, during storage under different a_w x temperature conditions.

Some research has been performed on the effect these factors have on individual fungal species. For example work by Luard and Griffin (1981) investigated the effect of water potential on fungal growth and turgor, and found that large positive turgor potentials were maintained in all species even when the external potential severely inhibited growth. Smith and Hill (1982) studied the influence of a_w and temperature on growth of *Aspergillus restrictus* and *A.versicolor* and found that *A.restrictus* grew faster than *A.versicolor* at lower a_w.

Gervais *et al.* (1993) determined the effect medium hydration had on the growth of *Trichoderma viride* and discovered that addition of silica gel to the medium proved to be limiting to fungal growth. More research needs to be concentrated on the interactions of fungi under various environmental conditions in cereal straw.

1.5 TECHNIQUES TO DETECT MYCOFLORA

In order to establish the rate of deterioration and thus losses in dry matter caused by the mycoflora on cereal residues, effective and efficient methods to detect the fungi are required. Different methods of assessing the mycoflora of cereal straw yield information on different aspects of colonisation and the use of two or more will often give complementary information. The choice of method employed should be governed by the nature of the investigation.

Lacey (1980a) investigated the microflora of straw and methods of assessment. He divided the methods into four areas; direct plating, direct observation, culture of washings and culture of spores suspended in air. Direct plating of tissue allows the proportion of pieces colonised by different species to be assessed. When preceded by washing or surface sterilisation (Sauer and Burroughs, 1986) it enables isolation of the fungi growing internally. Direct examination of fungi using light microscopy can provide much information on the types and distribution and the form of their growth on plant material when either incubated in a humid chamber or freshly harvested.

Washings of plant material and plating of the serial dilutions have been established for a long time, but there are many variations of the method. Dilutions may initially be prepared by shaking, pounding or maceration. The resulting suspension may be spread-plated on the surface of solidified agar media or pour-plated by mixing with molten agar then poured or microscopically examined. The problem with this method, is that efficiency of washing can vary with each method and cells may be killed by the heat of the molten agar (Flannigan, 1974). Also pipetting may not transfer material proportionally from one dilution to the next.

Culture of spores suspended in air can be achieved by two main methods. These are suspending spores in air in a sedimentation tunnel and trapping with a cascade impactor or using an Anderson sampler, which is particularly suitable for assessing species with spores that become easily airborne, especially actinomycetes.

Lacey and Dutkiewicz (1976a,b) have shown that the Anderson sampler is a more effective method for spore capture than the sedimentation chamber. Chang and Hudson (1967) were interested in the fungi of wheat straw compost and used the same techniques, except for the spore capture method. Hay has a similar mycoflora to wheat straw and the same methods have been employed by Lacey and Dutkiewicz (1976b).

Research by Flannigan (1978), Sandhu and Sindu (1980), Clarke and Hill (1981), Magan and Lacey (1985), Magan (1988a,b) and Robinson *et al.* (1994) have all used some of the methods discussed by Lacey (1980a), for the determination of

mycoflora from a variety of materials; including barley grain, wheat grain and bagasse.

A variety of alternative techniques to those previously employed have been developed by several researchers. Schnurer and Rosswall (1982) investigated using fluorescein diacetate (FDA) hydrolysis as a measure of total microbial activity in soil and litter. They found that spectrophotometric determination of the hydrolysis of FDA was a simple, sensitive and rapid method for determining microbial activity in soil and litter and that hydrolysis was found to increase linearly with soil addition for both *Fusarium culmorum* and *Pseudomonas denitrificans.*

Until research by Newell and Fallon (1991) there were not any available methods for the measurement of instantaneous fungal growth rates in field samples of crops. This information is essential for the study of microbial dynamics in decomposition systems. Newell and Fallon (1991) devised a technique for estimating instantaneous growth rates for ergosterol containing fungi in field materials. The method was based on measuring rates of radiolabeled acetate incorporation into ergosterol. This experiment was carried out using standing dead grass. Tothill *et al.* (1992) investigated the relationship between fungal growth and ergosterol in wheat grain and found that some correlation between microscopic and visible moulding, ergosterol and CFUs.

Respiration has been used to measure metabolic activity in stored produce for a long period of time, by a variety of researchers. Respiration rate is affected by water availability, oxygen consumption, temperature, microbial contamination,

mechanical damage, mite and insect infestation and the period and conditions of storage of the material, as demonstrated by a variety of researchers. The problem with total respiration values are that the two components; the degree of contribution of the fungal and material respiration remains controversial (Pomeranz, 1974). If cereal residues, such as cereal straws are used then the rate of respiration is due to the fungi present because the straw is a dead material. Respiration rates also vary with different cultivars, quality and age of material, period of the experiment and between the different methods of determining respiration. As dry matter is lost by the utilisation of carbohydrate during respiration, the data may also be used to measure dry matter loss.

At present there are a variety of methods employed to measure the rate of respiration. Hamer *et al.* (1991) used an innovative electrolytic respirometer designed by Tribe and Maynard (1989). This was designed to monitor respiration in soil and enabled continuous monitoring of oxygen uptake and measurement of total carbon dioxide production at different a_w levels and temperatures. Hamer *et al.* (1991) and Lacey *et al.* (1994) used the system to study respiration of cereal grains (wheat, barley and oilseeds) at different a_w levels and temperatures.

Beare *et al.* (1991) used the substrate-induced respiration (SIR) method for measuring fungal, bacterial and total microbial biomass on plant residues. The SIR method of Anderson and Domsch (1975,1978) was originally designed for use in soils and Beare *et al.* (1991) have optimised their method. The procedure involves short term measurement of respiration (4-5 hours) from soils following the addition of glucose. The rate of respiratory response over short time intervals was found to be proportional to chloroform-labile microbial biomass C.

Selective inhibitors, cycloheximide and streptomycin were added to soil sub-samples to define fungal and bacterial contributions, relative to the total glucose induced respiratory response. They believed SIR has several advantages; that it is simple and rapid, identifies active components of microbial biomass and allows separation of fungi and bacterial contributions to total respiration. The system, however, relies on induction of respiration and is thus not a true representation of natural decomposition, and environmental factors, e.g. a_w and temperature, have not been considered.

Gokhale and Isaac (1984) used enzyme assays, manometric and oxygen electrode techniques, to determine oxygen exchange and cytochrome oxidase activity for measuring respiratory activity in isolated protoplasts of *Aspergillus nidulans*. These procedures were designed for specific respiratory rates of individual fungi and not as a method for measurement of total respiration of a crop material.

More recently Robinson *et al.* (1993) investigated nutrient and carbon dioxide release by interacting species of straw decomposing fungi. They measured fungal respiration by infra-red gas analysis, which detects carbon dioxide release. The advantages of the respirometer system are that large samples can be tested, temporal measurements under different steady state a_w and temperature conditions are possible, monitoring of data is automatic and consistent results are obtained, as demonstrated by Hamer *et al.* (1991).

1.6 CURRENT USES OF WHEAT STRAW

In the United Kingdom the average amount of cereal straw remaining after a crop has been harvested is about 6 t ha $^{-1}$. As a result of this, over 12 million tonnes of cereal straw are produced annually and just under half of this is surplus to requirements (Hughes, 1979). Some of the remaining 50% of cereal straw was traditionally used by livestock farmers for feeding and bedding. In the USA there are 75 million tonnes of straw available annually with similar excesses. Straw is thus generally considered to be a waste product, because transport to potential sites of utilisation is expensive due to its bulk and its slow rate of decomposition relative to liquid substrates. Consequently transporting straw could only be economic if it can be converted into high value products.

Open-field burning or baling and removal were the major means of disposal prior to legislation banning burning in 1993. After this legislation, there has been a trend to switch to alternative methods of straw disposal, including chopping and shallow burying of straw in soil prior to sowing; in order to conserve the organic matter and prevent environmental pollution caused by straw burning.

The presence of the incorporated straw in the soil can hinder subsequent crop establishment mechanically, including blocking of the drill coulters. Problems may also arise biologically, by pathogenic micro-organisms colonising the straw in wet weather creating an anaerobic environment which can lead to the production of phytotoxic organic acids (Lynch, 1977; Harper and Lynch, 1981).

Research by Broder and Wagner (1988) investigated microbial decomposition of cereal residues in soil. They found that 47% of wheat residue decomposed over a

period of 32 days with the predominant fungi being *Trichoderma, Aspergillus* and *Penicillium* spp. This information is useful as the effects of incorporation of straw into soil need to be known so that optimisation of conditions can be achieved. An additional problem with incorporation of straw into soil is that it has been found that in southern Brazil some pathogenic fungi of economic importance can survive in surfaces of wheat residues until the next growing season (Fernandez, 1992).

Straw incorporation into the soil may also be beneficial, as it provides the major substrate input to the micro-organisms that inhabit the surface layers, and their beneficial activities include polysaccharide production which stabilises the soil. In the UK, however, the structural stability of soil is not a problem. In fact the presence of straw may account for up to 20% loss of grain after direct drilling in wet autumns (Lynch, 1983). In other countries incorporation of straw in soil is preferred to burning because the soils are structurally unstable and erosion by water and wind would otherwise occur if straw was removed.

At present the main use of excess wheat straw, apart from incorporation into soil, is composting of straw that provides the substrate base for edible mushroom (*Agaricus bisporus)* production. *A.bisporus* accounts for 75% of the total mushroom production. Usually wood is used as a substrate base for `oyster` mushrooms (*Pleurotus* spp.), however research by Kurtzman (1979) has shown that straw may also be utilised.

The UK mushroom industry uses only 200,000 tonnes of straw per year, a small proportion of the total straw produced. However, in 1979, the annual crop value

reached £56 million, exceeding the value of the tomato crop (MAFF, 1981). The production of mushrooms is increasing at an annual rate of about 10% per annum (Delcaire, 1978) and thus utilisation of straw in this industry has increased. The used compost may also be used as horticultural and agricultural fertilisers and soil conditioners.

A recent development is the use of wheat straw as a raw material for particle board manufacture. The first wheat straw particle board plant was established in July 1996 at Wawpeton in south eastern North Dakota, valued at $15 million (Anonymous, 1996). The equipment was supplied by Daproma System in Sweden, a company that provides keyboard plants for companies that use alternative materials such as bagasse, to Ed Shorma's cabinet and furniture business. The production goal of the plant is to contract with about twenty-five farmers to provide $3 million worth of straw annually and produce 1 tonne of $^{3}/4$ inch board for every 1.2 tonnes of straw.

1.7 POSSIBLE USES OF WHEAT STRAW

There are a variety of theoretical possible alternative uses for the excess wheat straw. These include a source of animal feed, a feedstock for fuel production, novel composts and use in the paper making industry. These will be discussed with reference to feasibility of production and most important of all, economic viability.

In order for straw to be used as an animal feed it has to be reduced to a finely divided state and / or it needs to be chemically pre-treated, usually with sodium

hydroxide or ammonia. The chemical treatment involves the reduction in size, addition of sodium hydroxide and pelleting; the resulting product is known as nutritionally improved straw (NIS). The patent for the NIS process was filed in 1971 and the first plant for production of 25,000 tonnes per annum opened in the UK at Kimbolton, Cambridgeshire in 1975 (Evans, 1980). The process needs to be carried out at manufacturing plants, therefore straw has to be transported to sites which adds to the cost. This process has been commercially accepted, although on-farm manufacture would be preferred.

An alternative to the chemical hydrolysis, which would be more environmentally friendly, would be to use micro-organisms. Semi-solid fermentation to produce animal feed has been achieved on straw. The semi-solid substrate was usually ryegrass straw, and as yet wheat straw has not been used. There are several advantages of semi-solid over submerged fermentation; the pH and temperature does not need to be rigorously controlled, no foaming occurs, aeration can be achieved by simple tumbling of the mass, no costly centrifugation is required and the absorptive properties of the material holds the nutritive substrates within the matrix which is made available to the micro-organisms directly. The cellulose decomposing fungi *Trichoderma viride* and white rot fungi have been used for this process. However, the technology involved is too complex for on-farm use.

Other simpler processes such as pre-treating barley straw with white rot fungi have been investigated (Latham, 1979) but the gains were small and would probably be uneconomical. Researchers in these areas have suggested a simple on-farm process that involves piles of straw which are ammoniated, inoculated and covered

with black polythene sheets in a sheltered area and after incubation the straw could be dried, pelleted and fed to animals.

At present the procedure has not been tested and evaluated on-farm and there are dangers of the possibility of mycotoxin residues which would be harmful to the animals. Therefore, further research would be required in these areas to eliminate risks to animals and make the process economically viable for the use of cereal straw.

The main fuel products that could be produced from microbial fermentation of straw are alcohol and methane. In Brazil sugar cane is used as a feedstock for the production of alcohol quite successfully. Cellulose in straw, however, is much harder to ferment than the sucrose in sugar cane and as yet there has not been any evidence to suggest that straw would provide a possible and economic feedstock. Further extensive research would be required before this could be a feasible and economic option.

Methanogenesis, which is the production of methane from anaerobic fermentation has been widely investigated for a range of agricultural wastes (Hashimoto *et al.*, 1980). Nevertheless little work has been performed on the possibility of wheat straw being used as a feedstock for anaerobic fermentative bacteria. The rate of digestion of straw is known to be slow and limited. Digestion rates may be increased if the straw is pre-treated with heat and chemicals. Unfortunately, this involves additional costs which may not make straw a suitable substrate for feedstock and not be viable economically.

In conventional composts, straw and animal wastes are mixed and the degradation process occurs naturally with little external control, resulting in a large variability in the quality of the end product. Due to these factors and economic reasons this is not a widespread use of straw. If compost quality could be easily and economically controlled, this may be a viable use of straw. Controlled environment composting is now being examined to efficiently control the process and produce a consistent level of quality.

Lynch and Harper (1983) found that by adding a mixed inoculum of a cellulolytic fungus and a nitrogen fixing bacterium onto wheat straw that the decomposition increased and it was envisaged that this technology could be applied on farm sites. However, trials in soil demonstrated that inocula did not increase the decomposition rates or nitrogen level significantly (Magan *et al.*, 1989).

Another possible use of this waste straw is its utilisation as a raw or constituent material for the paper making industry and is the main focus and overall aim of the DTI-Link project, as discussed later. About 10% of the fibre used to make paper world-wide each year is from nonwood plant fibres, including cotton, straws, canes, hemp and grasses. Non-vegetable fibres such as glass fibres and polyethylene are also used.

Straws from most edible grains are suitable for processing into pulp. The most important types are wheat, rice, barley and rye with yields typically about 35% for bleached grades to 65% for high yield pulps suited for linerboard or corrugating media. Straw has a low lignin content and due to this factor would be especially suited for fine papers.

The soda process is the most common method of pulping of straw and anthraquinone is sometimes used with soda or Kraft pulping of straw. Straw fibre lengths are on average 0.5-2.5mm with diameters of 0.01-0.2mm and the straw hemicelluloses are mainly xylans. These characteristics make straw pulp similar to hardwood pulp.

Nonwood fibre sources were used for hundreds of years before wood was used as a source for papermaking. In the USA on average paper contains only about 2% of nonwood fibres. Globally, however, the use of nonwood fibre is increasing faster than wood fibre; this may be due to bans on burning of cereal straw, public awareness for the need to preserve natural forests and the abundance of straw as a waste product which is a cheap and economical alternative to wood pulp. Another factor is that at present the UK paper and board industry imports about £600 million worth of wood pulp annually, therefore considerable savings could be achieved if straw could be used as an alternative. Interest is being shown by several major companies in using straw as a raw material for paper pulp production for corrugated and bleached paper. Experience from straw pulping mills in Europe have demonstrated that straw is an acceptable pulp for corrugated paper making, that fully bleached straw pulp provides desirable properties for high quality papers and unbleached pulp adds stiffness to fluting material.

In the USA all corrugated medium was made from straw prior to the 1930's. Around this time the chestnut blight made a lot of hardwood available, which was pulped successfully and made into corrugating board. By the end of the 1950's most of the straw-using mills were closed or converted into hardwood-using mills. At present almost all corrugating medium is made from hardwood and/or recycled

fibre in the USA. In contrast to this, Europe's largest corrugated medium mill based at Saica, in Zaragoza, Spain uses pulp from wheat, oat, rye and barley straw with secondary fibre. The mill produces 1200 tonnes per day of medium containing 25% or 50% straw pulp, with the higher grade containing the larger percentage of straw pulp. Saica plans to double its capacity and incorporate straw into linerboard in the early 1990's.

There are many factors that influence the suitability of raw material for the use in paper. These include the ease of pulping and yield of useful pulp, the availability and dependability of supply, the cost of collection and transportation of the fibre source, the fibre morphology, composition, strength, the presence of contaminants, such as silica and dirt and finally the seasonal nature of the supply, as storage to prevent decay is costly.

There are also problems that straw pulp mills can experience. These are that straw pulps have low drainage rates, so it takes more water and requires large washers which causes additional costs to the process. Chemical recovery of straw pulp liquors is complex and has not been practised until environmental pressures in the 1980's forced some mills to begin this practice. Since large quantities of water are required to wash pulps, large amounts of energy are needed to concentrate the dilute liquor from the brown stock washers. Fortunately about one half of the liquor can be pressed out of the pulp and undergo chemical recovery to avoid high evaporation costs. However, chemical recovery is interfered with by the silica content of the straw, although most of the silica is removed during alkaline pulping.

There are a variety of problems with using wheat straw as a pulp source and some of the reasons why the USA replaced nonwood fibres with wood are summarised in Table 1.1. The moisture content of paper has an important effect on paper quality; therefore, paper properties must be measured under standard conditions of relative humidity (50%) and temperature (23^0C). According to TAPPI T 402, paper should be placed in a hot, dry room (20-40^0C at 10-35% relative humidity) before placement in the standard room so that the moisture content of paper approaches its equilibrium moisture content (EMC) by absorbing water from the atmosphere. A slightly higher EMC would be achieved if the paper approached the EMC for a given relative humidity and temperature by giving off water due to the hysteresis effect.

It is important to know the rate of moisture loss or gain of paper to achieve equilibrium in order to understand how long to condition the samples. Manufactured paper immediately off the reel that has not been conditioned before testing may have a moisture content that is several percent below paper that has been conditioned in a standard room for an hour. Since properties of paper can significantly differ over a range of moisture contents, it would be expected that considerable problems in routine mill measurement and quality control of paper could be experienced.

Surprisingly, although the importance of controlling the relative humidity during paper making and the effect moisture contents have on paper properties has been known for some time, very little research has been done and the most recent and extensive publication giving paper properties as a function of moisture content was by Carson (1944).

Table 1.1 The Advantages and Disadvantages of straw as a fibre source

Advantages	Disadvantages
By-product from agriculture	Transportation and storage problems
Cheaper than wood, as is a waste product	Straws are bulky and contain silica
Large annual crop	Short harvest time of 1-2 months; thus heavy drain on capital
Needs little refining	Degrades quite quickly, so high losses
Makes excellent filler, good printing and smoothness	Low drainage rates and thus low production rates

Carson's research showed that for four types of paper the strength properties may vary by as much as 40% with relative humidity changes that are within the range of normal conditions. They also investigated the effect of folding endurance in relation to various relative humidities and found that fold values may change by a factor of five. Moisture content also has an effect on the curling tendencies of paper; papers tend to curl when each side of the paper is at a different moisture content.

There is as yet no detailed published information on the differences in yield and quality of pulp for paper production from different species of cereal straw in the UK. There has also been no investigation into the differences in yield and quality obtained between different varieties of the same species.

There are a variety of tests to determine the mechanical properties of paper. However, currently there are no methods for predicting pulp quality and yield from an analysis of the raw material; but as the demand for alternatives to wood pulp increase an investigation into the correct storage of the raw materials will become vital.

Research into correct storage of raw materials, such as wheat straw is scarce. At the present time mould-free straw has only been required for chicken litter and fibre board, which are relatively low value products. However, as high value products begin to be demanded the need for consistently high quality raw materials will be crucial.

There has been renewed interest by researchers into using wheat straw as a possible use of pulp for the paper industry. The removal of lignin from lignocellulosic plant material is essential for the production of high quality pulp. Usually sulphur containing chemicals are utilised for this process, however environmental awareness has led to the development of possible alternative techniques.

One area of research is the biological pre-treatment of the pulp by lignin degrading fungi, often referred to as biopulping and biobleaching. Research by Gionvannozzi-Sermanni *et al.* (1994) demonstrated that enzymatic treatments of wheat straw for short periods of time reduced the beating time and improved freeness. Both of these effects lowered the energy requirements by more than 50%. Since the lignin content of the straw is reduced after beating the chemical input for bleaching of the pulp can also be reduced. Thus this biotechnological approach results in easier pulping with less consumption of chemicals and energy.

Work by Jimenez *et al.* (1994) involved biological pre-treatments for bleaching wheat straw pulp. This research was instigated due to growing environmental concerns of the high polluting effects of lye wastes produced from chlorinated substances used in the process. Another approach is a chemical free pre-treatment process. Research by Kubikova *et al.* (1996) involved treating the straw biomass with hot compressed liquid water which resulted in almost all of the hemicellulose and up to 62% of the lignin being extracted from the lignocellulosic matrix. The remaining pulp consists of cellulose and residual lignin. As an additional benefit the water extract can act as a raw material, such as for fermentation and saccharification. Both areas of the research can be applied to the established

existing wood pulping industry, however in both cases research in these areas does not cover the initial quality of the straw. Lawther *et al.* (1996) have researched the effect of steam treatment on the chemical composition of wheat straw, particularly the importance of the polymeric components, which have the potential for development in a number of end-use industries.

At the present time there is little information on rates of spoilage and losses in quality of straw under different moisture and temperature conditions. The tolerance of spoilage and effects of quality for paper making have also not been investigated. Research by Wamukonya and Jenkins (1995) investigated the durability of wheat straw briquettes as a possible fuel for Kenya. They found that these briquettes were the least durable and expanded the most, compared to sawdust briquettes. However, a combination of the two materials improved durability. They concluded that further analysis and economical considerations would have to occur before this was a viable option.

Fuels from straw have proved extremely difficult to burn in most combustion furnaces, particularly those designed for power generation. Significant maintenance problems of straw biomass for energy occur, for example the rapid formation of unmanageable deposits comprising of complex silicates, sulphates and carbonates on fireside surfaces. This leads to reduction in facility efficiency, capacity and increased costs (Jenkins *et al.*, 1996). To date none of the biomass power plants built in California can economically fire straw. In Denmark straw burning for fuel has been more successful, and more recently in the UK a new plant in Ely, Cambridge has been commissioned for this purpose (Gant, 1996). As

this electricity plant is in the early stages of production its success has yet to be determined.

1.8 USE OF BIOCIDES IN THE PAPER INDUSTRY

Biocides are used in the paper industry quite extensively to control the activity of micro-organisms, particularly bacteria and fungi. There are three main problem areas with microbial growth as shown in Table 1.2. These micro-organisms grow around the paper machine and produce slime that consists of polysaccharides and proteins. This slime may break off in pieces and lead to actual holes in lighter weight papers, pitting of paper, and even breaks in the web which lead to very expensive downtime.

A number of fungi and bacteria will grow in various raw materials used to make paper including wheat straw. In order for micro-organisms to grow, the correct pH, temperature, water availability are required. As a rule the pH range is 2.5-8 for fungi and pH 6-8 for bacteria and the optimum temperature, generally 25^0C. Some anaerobic micro-organisms grow in conditions where oxygen is absent and produce hydrogen sulphide, methane, or hydrogen, which have caused fatal explosions at mills. The sulphate using bacteria can also cause considerable corrosion at mills. Microbial growth may be increased by using recycled fibre, large amounts of starch and poor housekeeping.

Slime production may be controlled by good housekeeping and the use of biocides. The quantities of biocides usually added are from 0.05-1 kg per tonne of paper. There are two main types of biocides used, oxidising and organic biocides.

Table 1.2 The problems with microbial growth in a pulping company.

Area	Problem
Production	Slime deposits:- Breaks Corrosion Felt plugging Odours Reduced flows
Quality	Holes and Spots:- Sheet odour Machine aesthetics Discoloration & Brightness losses Increased dirt count
Raw Materials	Fibre degradation:- Additive contamination & Odour Reduction in strength properties Coating mass deterioration Fouling of probes

Oxidising biocides include chlorine or chlorine dioxide. In papermaking systems below pH 6 chlorine exists as the highly effective HOCl species and above pH 9 the active species is OCl^- which is less effective. Chlorine dioxide is added at 500-2000 ppm at various stages of the process, although it quickly disappears from the system.

There are several organic acids used and these include quaternary ammonium salts, methylene bis-thiocyanates, brominated propionamides, carbamates, glutaraldehydes and finally the isothiazolins. Quarternary ammonium salts which contain alkyl, aryl or heterocyclic substituents of C_8 - C_{25} are most effective under alkaline conditions, although they lose their effectiveness when lots of contaminants are present.

Methylene bis-thiocyanates are effective against sulphate reducing bacteria of the *Desulfovibrio* genus. However, they decompose in whitewater systems if the pH is above 8. The brominated propionamides are broad spectrum biocides that are extremely potent. Carbamates are effective above pH 7 and occur as dialkyl or monoalkyl and those containing short chain alkyl groups are more toxic than long alkyl chains. The glutaraldehydes may react with the proteins of the micro-organisms and has two reactive aldehydes that readily react with amines.

Finally the isothiazolin group has an acute and broad activity, due to its mixture of two compounds. Sometimes two or more biocides are used together to produce a synergistic effect against the micro-organisms. At present there has been very little research into the effect biocides have on controlling fungal growth on wheat straw, let alone their effect on wheat straw used as a possible pulp source for

papermaking. Initial research into the effectiveness of controlling microbial activity using chemicals was by Lacey (1974), who was interested in controlling and preventing moulding of sugar cane bagasse using propionic acid.

Work by Easson and Nash (1978) investigated the preservation of moist hay again using propionic acid and later, research by Lord and Lacey (1978), Lord *et al.*(1981), Lacey *et al.* (1981), and Magan and Lacey (1986a,b) all investigated the effects of propionic acid on hay under various conditions. However, these chemicals are all fungistatic; i.e. they only inhibit the growth of fungi on contact and they may subsequently grow should the acid be lost by evaporation. Thus their effectiveness depends on efficient coverage of the substrate. Another problem with using propionic acid, apart from its toxicity to handlers, is that research by Lord *et al.* (1981) showed that from measurements of the disappearance of propionate from cultures of *Paecilomyces variotii* and *Eurotium amstelodami* that these species could metabolise propionic acid and thus allow other species to colonise the substrate, especially in under-treated pockets of hay.

Until research by Magan and Lacey (1986a) there was little knowledge of the ability of other organisms to tolerate or metabolise propionic acid-based preservatives. They found that the yeasts *Candida guilliermondii* and *Hyphopichia burtonii* were able to grow on and metabolise more than 100 mmol litre^{-1} of ammonium propionate, which was a similar concentration to that used by Lord *et al.* (1981). However, very few studies have considered preservative concentration x water availability effects on growth / inhibition of such spoilage fungi.

Some research into the use of alternative biocides to propionates have been performed by several researchers (Grossbard and Harris, 1977). They investigated the effectiveness of the herbicide Gramoxone W in soil with respect to straw decay and found that it frequently delayed its decomposition. However, the effect on control of fungal growth on stored straw was not considered.

Lacey *et al.* (1981) investigated over one hundred different chemicals for preventing moulding of damp hay and found that fewer than a third showed any promise as a preservative and none met all of the criteria. However, 8-quinolinol showed some potential as an additive to propionate. Sulphur dioxide has been tested for its ability to control fungal growth on wheat grain. However, after an initial decrease in growth, for twenty eight days, little difference occurred when compared with control, so the threshold limits need to be carefully chosen (Magan, 1993). Research would have to be undertaken to establish whether this was a viable option for control of fungi on cereal straw.

At the current time there has been no research into the effect biocides have on cereal straw fibre quality, texture and tensile strength. This information will be essential if the excess straw is to be used as a pulp source for papermaking. The paper industry has criteria for fibre quality, texture, tensile strength, colour and yield which they require. However, the effects of fungal spoilage have on these factors is to date unknown. It is vital that this information is determined, so that a consistent high quality raw material can be provided for the paper making industry.

1.9 AIMS AND OBJECTIVES OF RESEARCH

The main aim and objective of the project is to determine which economically viable treatments best preserve the value and quality of straw during storage. At the present time there is very little quantitative data on how rapidly microbial growth occurs in relation to the condition of the stored straw, what effect these micro-organisms have on straw properties and consequently the value of the straw and how microbial growth may be restricted and controlled by systems that will be feasible and economically viable. This project aims to answer these questions and ultimately to provide a consistently suitable high quality straw for the use as an industrial raw material. This raw material will hopefully be used to produce high value products which will be in demand by industry. It is estimated that the typical annual usage of straw may be 50,000 tonnes per processing plant and systems would need to be developed to ensure that a bulk store of 5,000 tonnes retains a consistent quality for the end use.

The problems with achieving a consistent standard of straw is that the extent of microbial growth within a bale is not easily observed from the exterior and conventional harvesting and storage methods can, especially during poor weather conditions, result in moulding and microbial degradation of the straw before it reaches the processing plant. This causes problems including difficulties in handling and breaking of the mouldy sections of the bales. It may also reduce fibre strength, pulp yield and problems with discoloration of unbleached pulp and / or increased bleaching costs if used in papermaking. A further problem may be that unacceptable levels of mould spores are produced, which operators are then exposed to.

Economic storage and transport could only be achieved if the cereal straw is compacted into large, high density bales. Unfortunately, large, high density bales are not conducive to good storage. This is especially the case when the straw has been baled too moist, because air movement and thus loss of moisture is very slow. Currently, the effects of moisture content, package density and crop temperature on microbial activity are not fully understood and without this quantitative information the best storage conditions cannot be determined. The cereal straw may be used for a variety of end uses, although the main area of interest is to use wheat straw as a possible pulp source for papermaking.

The overall objectives of this thesis are:-

1. To isolate and identify the mycoflora of Winter wheat straw. The cultivars investigated were Ribband 1994-1996 and Beaver 1994.

2. To determine fungal succession on straw stored under various a_w x temperature interactions and quantify the associated dry matter losses.

3. To determine the effects of various a_w x temperature interactions on the rate of microbial spoilage.

4. To calculate the extent of spoilage and colonisation of the straw using respiration equipment under various environmental conditions.

5. Determine the efficacy of various biocides on the natural mycoflora and individual dominant fungi in vitro and on straw.

6. Conduct a series of small and large scale field experiments to determine the effect of the best biocide treatments on control of fungal spoilage under different moisture contents and bale densities.

CHAPTER 2

GENERAL METHODS

&

MATERIALS

2.1 PREPARATION AND STORAGE OF WHEAT STRAW

Two cultivars of winter wheat, Beaver (1994) and Ribband (1994, 1995 and 1996) were used in this study. In 1994 the cv. Beaver was obtained from a local farm in Newport Pagnall. The Ribband cultivar was used by all of the research partners, including Silsoe Research Institute (SRI) and Scottish Crops Research Institute (SCRI).

The wheat straw was chopped with Wilkinson sword secators into approximately 10mm lengths for microbiological analysis and respiration studies. The straw was stored in a cold room at 4^0C with less than 14% (wet basis) water content after harvest, so that fungal growth could not occur. When analysing for microbial contamination samples were handled inside a microbiological safety cabinet.

2.2 DETERMINATION OF THE MYCOFLORA OF WHEAT STRAW

The following general media were used in this study :-

(a) 2% Malt extract agar (MEA)

Distilled water	1L
Malt extract agar (Oxoid)	50g
Technical Lab M agar no. 2 (Oxoid)	5g
Chloramphenicol	5mg

(b) 10% Malt salt agar (MSA) (For xerophilic fungi)

Distilled water	1L
Malt extract agar (Oxoid)	50g
Technical Lab M agar no. 2 (Oxoid)	5g
Sodium chloride (NaCl)	100g
Chloramphenicol	5mg

(c) Czapek Dox agar (CZA)

Distilled water	1L
Czapek Dox agar (Oxoid)	45.4g

(d) Potato Dextrose agar (PDA)

Distilled water	1L
Potato dextrose powder (Oxoid)	50g

(e) 0.1% Agar diluent

Distilled water	1L
Technical Lab M no. 2 agar	1g

(f) Wheat straw extract agar (WEA)

Chopped straw (20g) was boiled in 1 litre of distilled water for 15 minutes. The extract was passed through muslin to exclude straw fragments and made up to 1 litre with distilled water (Magan, 1988b). The stock solution was diluted 1:1 with distilled water to produce 1% straw extract broth.

The pH of straw extract was adjusted to pH 5.5 with 0.2M hydrochloric acid. Technical agar no.2 (Lab M 15g) and 5mg of an antibacterial agent (Chloramphenicol) was added to straw extract, prior to autoclaving.

(g) 2% Milled straw agar

Milled straw (10g) was added to 50g of Technical agar no.2 (Lab M) and dissolved in 1 Litre of distilled water. Chloramphenicol (5mg) was added as before and the medium autoclaved. All media used in this study was autoclaved at 121^0C for 15 minutes.

(h) Culture and Suspension media

Fungi were isolated and cultured on agar media in plastic 9cm Petri dishes, containing approximately 20ml of media. 2% MEA slopes in Universal bottles containing approximately 10ml of media were used to store pure cultures and kept at 0-4^0C. Species of fungi were transferred to new MEA slopes every six months to maintain viability.

2.2.1 Adjustment of water activity (a_w) in media

Glycerol solutions were used to adjust 2% MEA in the range 0.75 - 0.95 a_W (Dallyn and Fox, 1980). Glycerol solutions were prepared according to the formula :-

$$G = \frac{(MW \times MC \times S)}{1000}$$

Where G = weight of glycerol (g)

MW = molecular weight of glycerol (92.09)

MC = molal concentration of glycerol

S = weight of solvent (g)

Table 2.1 demonstrates the molarity of glycerol solutions used to control equilibrium relative humidities (ERH) in media.

2.2.2 Adjustment of pH in media

MEA (2%) was adjusted in the pH range 4.4-6.4 in 0.2 unit increments. For adjustments between pH 4.4-5.6 McIlvaine's buffer (1921) was used. In the range pH 5.8 - 6.4 the buffer of Gomori (1955) was used. Tables 2.2 and 2.3 respectively detail the method of preparation of the buffers.

Table 2.1 Molarity of glycerol solutions used to control equilibrium relative humidities (ERH) in media (Dallyn and Fox, 1980).

Water Activity	Molarity of Glycerol
0.75	15.00
0.80	11.60
0.85	8.50
0.90	5.50
0.95	2.50

Table 2.2 Citric acid - Na_2HPO_4 buffer solutions used to modify the pH of media in the range 4.4-5.6 (McIlvaine, 1921).

PH	x ml 0.1 M citric acid[1]	y ml 0.2 M Na_2HPO_4[2]
4.4	55.90	44.10
4.6	53.25	46.75
4.8	50.70	49.30
5.0	48.50	51.50
5.2	46.40	53.60
5.4	44.25	55.75
5.6	42.00	58.00

1.Citric acid monohydrate, C6H8O7. H2O, M.wt. 210.14; 0.1 M solution contains 21.01g / L(x).

2.Na2HPO4, M.wt. 141.98; 0.2 M solution contains 28.40g / L(y).

x ml 0.1 M citric acid and y ml 0.2 M Na2HPO4 were mixed.

Table 2.3 Na_2HPO_4 - NaH_2PO_4 buffer solutions used to modify the pH of media in the range 5.8-6.4 (Gomori, 1955).

PH	x ml 0.2 M Na_2HPO_4[1]	y ml 0.2 M NaH_2PO_4[2]
5.8	4.0	46.0
6.0	6.15	43.85
6.2	9.25	40.75
6.4	13.25	36.75

1.Na_2HPO_4. $2H_2O$, M.wt. 178.05; 0.2 M solution contains 35.61 g / L.

2.NaH_2PO_4. $2H_2O$, M.wt. 156.03; 0.2 M solution contains 31.21 g / L.

x ml Na_2HPO_4. $2H_2O$, y ml NaH_2PO_4. $2H_2O$; diluted to 100 ml with water.

2.3 ISOLATION AND ENUMERATION OF MYCOFLORA

2.3.1 Direct Plating

Initially the size of the pieces of straw to be used was determined from which species would develop and be enumerated easily. Wheat straw pieces were cut into 1mm, 3mm, 5mm, 10mm and 15mm lengths. Five pieces of each length were placed aseptically onto each of three MEA and MSA plates. These plates were incubated for 7 days at 25^0C (King *et al.*,1984). Straw segments (1g) were also washed in 9ml of 0.1% agar diluent and shaken for 1 minute. The straw was removed aseptically using tweezers and dried on Whatman filter paper No.1. A total of 5 washed pieces were transferred onto MEA and MSA and incubated for 7 days at 25^0C. All procedures were carried out in a laminar flow cabinet.

To determine the internal mycoflora wheat straw pieces were shaken for 20-30 seconds in 95% ethanol, then transferred into 1% NaOCl and left for 1 minute. The straw pieces were rinsed with sterile water, dried and direct plated, 5 pieces per Petri plate. The three replicates were incubated for 7 days at 25^0C (Sauer and Burroughs, 1986). The fungi growing from the straw pieces were enumerated and the frequency of isolation of individual and total species determined.

2.3.2 Dilution plating

Sub-samples of chopped straw (1g) were added to 9ml of 0.1% agar diluent and shaken for 1 minute. A serial dilution series was prepared and 0.1ml of diluent was spread-plated with a glass spreader onto three replicate agar plates (Pitt, 1988;

Magan, 1988a,b). All pipetting was carried out with 1ml and 200µl automatic pipettes (Gilson) with sterile disposable tips. Plates were incubated for 7-10 days before enumeration.

2.3.3 Identification

Fungi isolated from straw were identified by examination of the following characteristics :-

(a) Microscopic observations of the ultrastructure.

(b)Recording colony characteristics, including colour, texture, reverse pigmentation and presence of exudate.

(c) Size of colony in millimetres.

Fungi were classified to genus level using standard texts (Samson *et al.*, 1981) and to individual species of *Aspergillus* (Raper and Fennell, 1965; Klich and Pitt, 1988), *Fusarium* and *Penicillium* (Pitt, 1988; Christenson *et al.*, 1994). The colonies were then subcultured onto specialised media including MEA, CZA and PDA to aid in identification by enhancing growth characteristics and sporulation.

2.3.4 Storage of Penicillium *species.*

Spores from 14 day old colonies of *Penicillium* species were removed using a sterile needle and inoculated into sterile bijou bottles containing 0.5ml of 0.1%

agar diluent. The solutions were mixed and the contents stored at 0-4^{0}C (Magan, 1993).

2.3.5 Subculturing of species

The edge of growing mycelium from species on agar, after 7 days, was removed using a 3mm diameter cork borer and the plugs centrally transferred onto selective media, such as CZA, MEA and WEA. The plates were incubated at 25^{0}C for 4 days. For subculturing *Penicillium* species, a sterilised loop 3mm diameter was used to remove the spore suspension and centrally inoculate agar. The plates were incubated at 25^{0}C for 4 days.

2.4 ISOLATION OF DOMINANT FUNGAL POPULATIONS FROM WHEAT STRAW

2.4.1 Effect of Temperature, a_w and pH on growth of dominant fungi isolated from straw

The wheat straw cultivar used in this experiment was cv. Beaver (1994) and cv. Ribband (1994,1995,1996). Straw pieces were prepared as described in section 2.1. Direct plating and serial dilutions of straw pieces (5 per plate) were inoculated onto pH adjusted media in the range 4.4-6.4, with three replicates per treatment. The plates were incubated for 7 days over the temperature range 10^0-30^{0}C. The procedure was repeated using a_w adjusted straw pieces plated onto adjusted media in the range 0.75-0.98 and incubated over the same temperature range.

2.4.2 In Vitro Studies under a range of Temperature, a_w and pH conditions

The effect of temperature (10-30^{0}C), pH (4.4-6.4) and a_w (0.75-0.98) on the growth of dominant straw fungi was determined in vitro on pH/a_w adjusted media. The species used in this experiment were the dominant species determined from the results in 2.4.2. The species used are shown in Table 2.4.

Agar plugs were removed from the growing margins of colonies of each species after 7 days of growth, using a 3mm diameter cork borer, except for *Rhizomucor*, *Aspergillus* and *Penicillium* species. The plugs were placed in the centre of pH or a_w-adjusted media and incubated for 7 days. For the *Penicillium*, *Aspergillus* and *Rhizomucor* spp. spore suspensions were first prepared and using an inoculating loop with a diameter of 3mm the solutions were centrally inoculated onto the media. All experiments were carried out with three replicates per treatment. The colonies were measured in two directions at right angles to each other daily for 7 days. Growth rates were determined over the temperature range 10-30^{0}C.

2.4.3 Determination of Moisture Content, a_W and Adsorption Isotherms

Samples of chopped straw (1g) were weighed in 25ml glass screw top bottles. A known amount of water was added to hydrate the samples, the bottles closed, shaken and allowed to equilibrate for 24 hours at 4^{0}C. The bottled samples were then transferred to the desired temperature for 4 hours. Three replicates of each treatment of moisture content were transferred to glass beakers and placed in the oven at 115^{0}C for 24 hours.

Table 2.4 Dominant species isolated from wheat straw.

Species
Alternaria alternata (Fr.) Keissler
Aureobasidium pullulans (de Bary) Arnaud
Cladosporium cladosporioides (Fres.) de Vries
Epicoccum nigrum (Link)
Eurotium amstelodami (Mangin) Thom and Church
Fusarium culmorum (W.G. Smith) Sacc.
Rhizomucor pusillus (Mich)
Penicillium aurantiogriseum (Dierckx) Stolk and Samson
Penicillium hordei (Stolk)
Sporobolomyces roseus (Kluyver and Van Niel)
Trichoderma harzianum (Rifai)

Samples of straw were reweighed after cooling and the moisture content calculated using the formula :-

$$\text{Moisture content} = \frac{F - D}{F} \times 100$$

where F = Fresh weight (g)

D = Dried weight (g)

Water activity was determined by placing sub-samples of straw from the above treatments and placing into the humidistat chamber (Humidistat IC II, Novasina A.G, Switzerland). The humidistat chamber was maintained at the temperature investigated (10-30^{0}C) and the sample allowed to equilibrate. The readings were recorded until no change in value was obtained, this was usually after 4 hours. The a_w during adsorption of different amounts of water was determined for the straw in order to determine the quantities of water necessary to hydrate straw for use in subsequent experiments.

Distilled water was added in volumes of 0-0.4 ml in 0.01 divisions per gram of straw, allowed to equilibrate and the moisture content and a_w determined as previously explained. The adsorption isotherms of straw were determined for 10^0-30^{0}C by plotting a_w against moisture content values. The moisture content for a given a_w could then be obtained from the fitted curve.

2.5 EFFECT OF ENVIRONMENTAL FACTORS ON FUNGAL RESPIRATION ON STRAW

2.5.1 Respirometer System

A respirometer system designed by Tribe and Maynard (1989) consisted of glass leaching tubes (280 x 27mm) sealed with rubber bungs at each end and contained two fitted glass tubes and the electrolysis cell and sample within this system. This system was not ideal as the bungs had to be sealed with silicone to maintain air tightness, and had to be resealed when opening the system to change solutions.

This system would only allow about 5g of straw to be used which was not adequate for our purposes. The system was modified by using 500ml glass Duran bottles enabling 20g (dry weight) of chopped straw to be used. Plate 2.1 showed the schematic representation of one respirometer unit (see Appendix for modified system). The screw top caps had two holes 6mm x 6mm, which were 10mm apart. Inserted into these holes were the existing glass tubes used by Tribe and Maynard (1989), and these tubes were maintained in their positions by sealing with Araldite™ around the inner and outer surface between the cap and the tube, this also achieved an adequate airtight seal.

The existing polypropylene Y piece compensator bottle and Suba Seal caps were attached to the glass tubing. The internal assembly of the electrolysis cell and sample were the same as the old system, the only difference was that larger volumes of sample could be held in each respirometer unit.

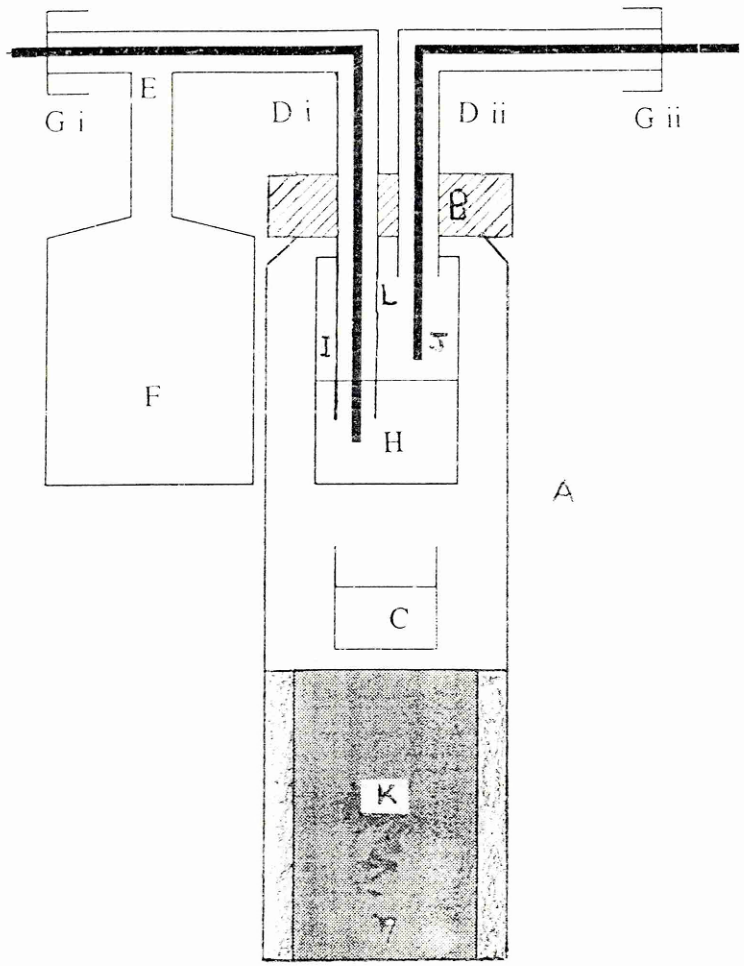


Plate 2.1 Schematic representation of one respirometer unit.

A) Duran bottle (500ml); B) plastic screw lid fitted with two glass tubes; C) alkali vessel (Sterilin polystyrene bijou tube) resting on sample; Di) 110 mm glass tube, 10 mm above lid, 75 mm below lid, under electrolyte surface; Dii) 50 mm glass tube, 20 mm above lid, projecting 20 mm outwards; E) polypropylene Y-piece; F) compensator bottle; Gi,ii) Suba Seal caps through which the electrode wires are threaded; H) electrolysis cell (Sterilin polystyrene test tube) containing acidified $CuSO_4$ electrolyte; I) cathode of copper wire; J) anode of platinum wire; K) straw sample; L) hole in cell cap.

2.5.2 Effect of temperature and a_w on fungal respiration

For each experiment 15-30 respirometer units were used, consisting of various moisture contents with three replicates of each. Each unit was placed in a water bath container that had specially designed clips to hold the bottles in place, once the water bath was filled. It was possible to use a constant temperature room for some of the experiments, such that the bottles were held in the clips without any need for water. Straw was modified to 0.75-0.98 a_w by addition of known amounts of water calculated from the moisture sorption isotherm curves. The temperatures used to investigate the effect on respiration were 10-30^{0}C with three replicates for each treatment and the experiments repeated twice. The experiments were run for 14 days and solutions changed when necessary.

2.5.3 Enumeration of fungal species from respiration studies

At the end of each experiment sub-samples of straw from each respirometer bottle were assessed for visible moulding and inoculated onto glycerol-adjusted media, the same a_w as the test conditions, for determination of the range of mycoflora present using direct and dilution plating. The remainder of each sample was frozen at -40^{0}C and sub-samples sent to SCRI for lignin and cellulose analysis. Details of the methods used by SCRI are in Section 2.5.4.

2.5.4 Materials and methods of work carried out at SCRI

Preparation of straw samples

Upon receipt, the cereal straw samples (100 g) were dried in an oven at 50^0C before being milled in a hammer mill to pass a 1 mm screen.

Determination of lignin contents

The lignin content of all straw and stored straw samples was determined by the acetyl bromide method (Morrison, 1972 a,b) as modified by Iiyama and Wallis (1988). The milled straw samples, in triplicate, were dissolved in 25 % acetyl bromide in acetic acid (v/v) containing 4 % perchloric acid (70 % v/v) by heating at 70^0C for 30 minutes. After diluting to a suitable volume with NaOH/acetic acid, the absorption of the solution was determined at 280 nm and the lignin content determined from known standards.

Determination of non-cellulosic polysaccharide (NCP) contents

Straw samples (100 g) were weighed into glass vials and heated with water at 70^0C for 30 minutes to dissolve the small amount of sugars arising from the cell contents. After centrifuging and drying, the samples were then heated with 2 M trifluoroacetic acid (TFA) at 121^0C for 1 hour to hydrolyse the NCP to their constituent monosaccharide residues. After centrifuging, aliquots were removed and their carbohydrate content was determined by the phenol-sulphuric acid method (Dubois *et al.*, 1956). The standard used was D-xylose, the predominant neutral sugar present in the NCP fraction of cereal straw. The cellulose component of the straw is not soluble in 2 M TFA.

Infrared spectra

Infrared spectra of straw and treated straw samples were recorded on a Bruker IF66 infrared spectrometer using a Diffuse Reflectance Infrared Transmission (DRIFT) cell (Graseby Specac UK Ltd). The spectra were recorded over the range 4000-400 cm^{-1}, 500 interferograms being recorded for each sample, and the data were processed by the vector normalisation procedure. All spectra were background subtracted using KBr as the background. Plate 2.2 showed the DRIFT spectroscope equipment used for analyses of the straw samples at SCRI.

The DRIFT cell, shown in Plate 2.3 allows fibres to be analysed without any prior preparation. The sample (100 g) is simply cut into reasonably small pieces then placed into the sample cup (C). The sample is then levelled of with a spatula and analysed directly. For samples as coarse as chopped fibres, the background is run against a sample cup filled with Potassium bromide (KBr), or even better, a small piece of fine grade sandpaper that has been sputter-coated with aluminium (Bruker, UK; Personal communication). The incident IR radiation from the laser (A) is focused by mirror (B) on to the sample cup (C). When this encounters the sample, the surface heterogeneity causes the unabsorbed, or reflected, radiation to be scattered. This is focused by the second curved mirror (D) and directed to the detector (not shown). Although the detected radiation can often be weak, the use of FT allows the accumulation of multiple scans, improving the quality of the resultant spectrum.

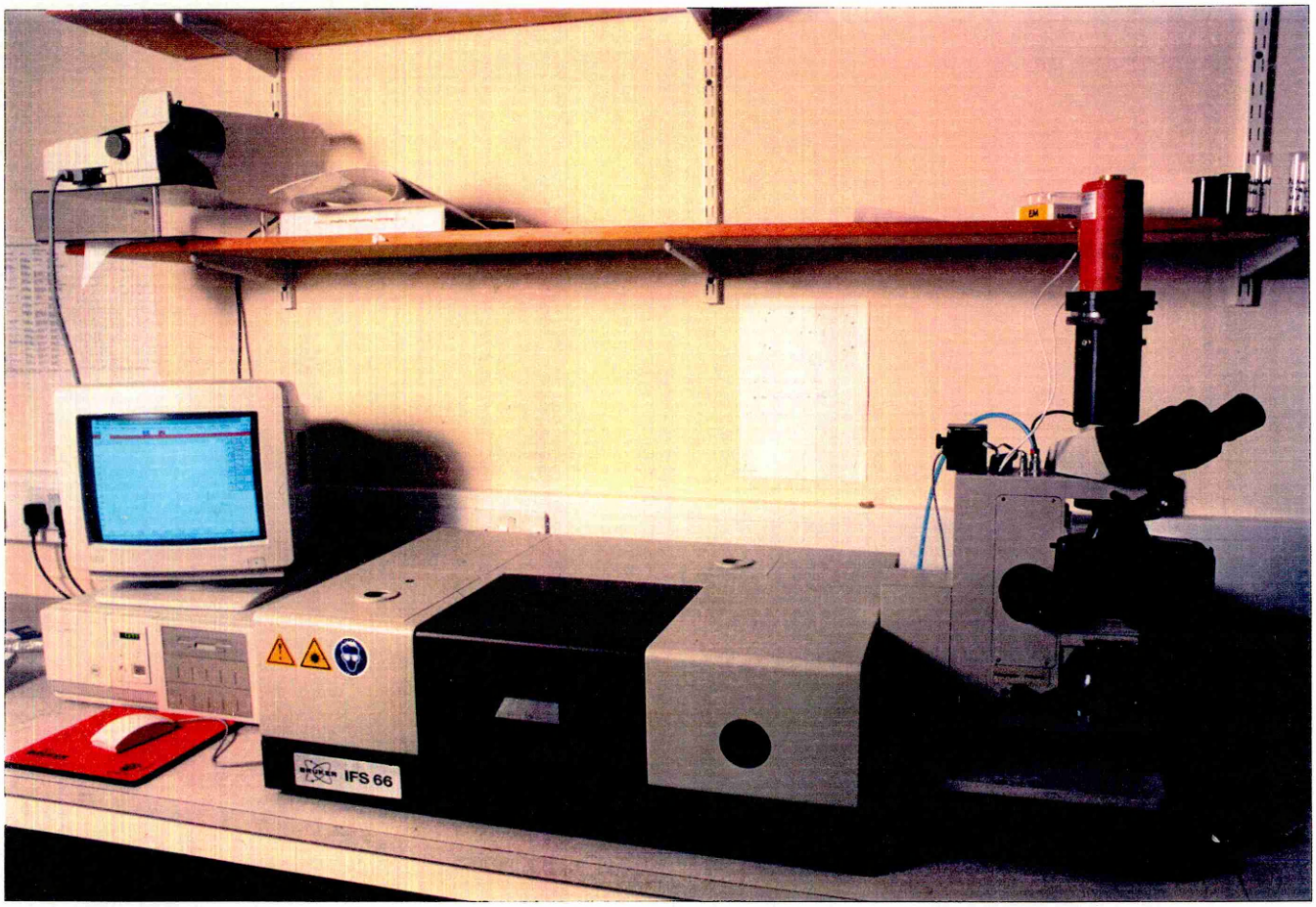


Plate 2.2 The DRIFT spectroscope used for fibre analyses at SCRI.

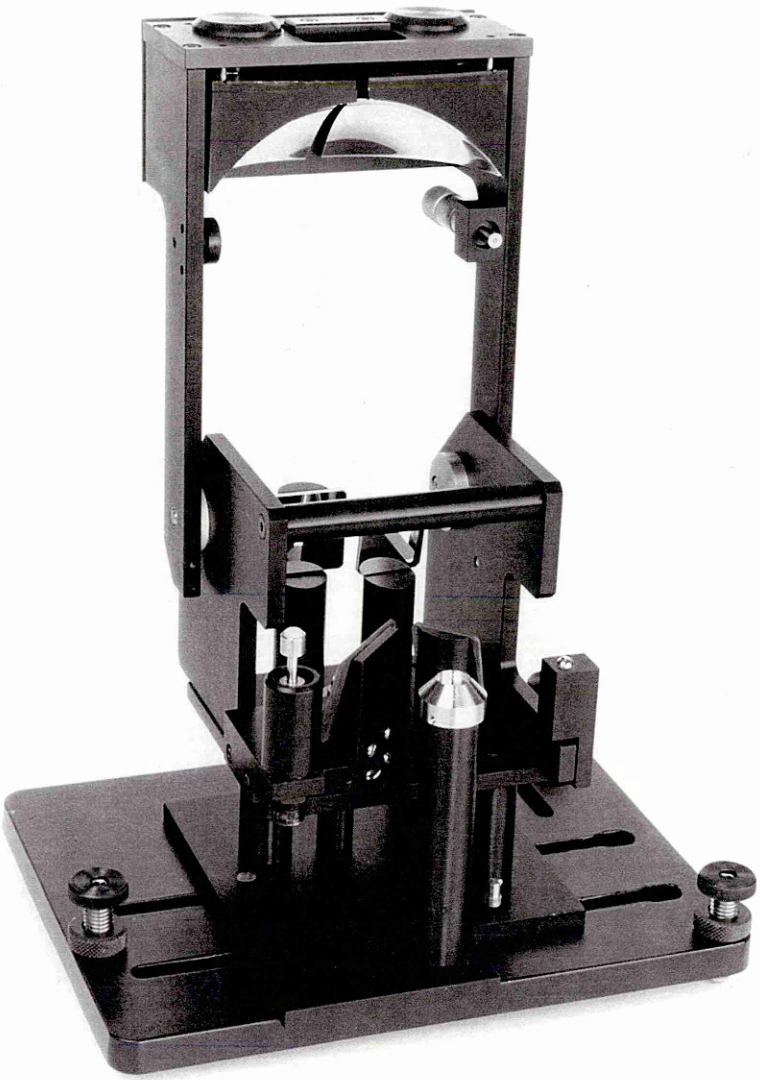


Plate 2.3 The DRIFT cell used by SCRI.

2.5.5 Measurement of respiratory activity of component fungal species on straw

The respiratory activity of *A.alternata*, *C.cladosporioides*, *F.culmorum*, *E.amstelodami* and *P.aurantiogriseum* were compared by inoculation of autoclaved sterile straw modified to 0.80-0.98 a_w over the temperature range 10-25^{0}C using the respirometer system. Agar plugs (5 per bottle) were removed from the growing margins of colonies of each species after 7 days of growth, using a 5mm diameter cork borer after the a_w of the straw had equilibrated for 24 hours. The agar plugs were weighed to determine the quantity of mycelia present. The experiments were run for 14 days and solutions changed when necessary.

2.5.6 Electrolysis units for measuring of oxygen consumption

For each respirometer cell, 100g $CuSO_4.5H_20$ were dissolved in 160ml 1 M H_2SO_4 at 75^{0}C and about 9ml were distributed into each electrolysis cell when temperature was 60-70^{0}C. When the electrolysis cell was assembled the platinum anode tip was positioned slightly above the electrolyte meniscus. The standard charge of electrolyte in each cell had the capacity to produce approximately 175ml oxygen (dry NTP) at 25^{0}C.

2.5.7 Carbon Dioxide determination

The carbon dioxide evolved during respiration was absorbed into 5ml aliquots of 2M sodium hydroxide solution in the tubes placed on the straw surface. Each tube had the capacity to absorb 220ml carbon dioxide (NTP). Sodium hydroxide tubes were replaced with new solutions when the tubes became cloudy in appearance indicating saturation of carbon dioxide. Sodium hydroxide solutions were removed

from the tubes and made up to 50ml with distilled water in volumetric flasks. The solutions (10ml) were titrated with standardised 2M HCL dispensed from a 10ml automatic burette (BDH) while stirring. The volume of acid required to adjust the solution to pH 8.3 indicated by a colour change of purple-red to colourless by the indicator 0.5% w / v phenolphthalein (BDH) in 95% ethanol was recorded. The solution was then titrated to pH 4 using a drop of methyl orange indicator (BDH) which changed colour from green to blue-grey.

The volume of carbon dioxide absorbed by the NaOH was calculated using the formula:-

V_{CO2} (dry, STP)=$(V_2-V_1-(C_2-C_1))$x 22.44

where V_{CO2} = volume (ml) of dry carbon dioxide absorbed at STP

V_1 = volume (ml) HCL to pH 8.3 (sample)

V_2 = volume (ml) HCL to pH 4 (sample)

C_1 = volume (ml) HCL to pH 8.3 (control)

C_2 = volume (ml) HCL to pH 4 (control)

2.5.8 Data Sampling

When the respiratory system was set up the electronic control unit (ECU) was switched on to supply current to each respirometer unit. The current flow in electrolysis cells were measured by a four channel multiplexed ammeter inside ECU and recorded with a BBC master series microcomputer with dual disc drive

and monitor. The software for the BBC disc filing system (DFS) contained three programs to run the data sampler and plot or print data files. The program floppy $5^1/4$" disc and an empty formatted $5^1/4$" disc for data collection were inserted into the dual disc drives.

Histograms were displayed on the monitor screen which showed respiration characteristics of the units at any one time. Data was recorded every 60 minutes for 14 days. At a later date the system was modified so that an IBM computer could be used to record the data.

2.6 EFFECT OF a_w AND TEMPERATURE ON FUNGAL SUCCESSION ON STRAW

To compliment the respiratory work, the effect of a_w x temperature interactions on the fungal succession and dry matter loss on straw was monitored over a period of 6 months. The method consisted of weighing 1g samples of chopped straw into 10ml glass bottles, three replicates per microbial analysis and three for dry matter loss determination for each removal date (2, 4 and 6 months).

The straw samples were modified in the range 0.85-0.98 a_w and stored at the temperatures 10^0C, 15^0C and 25^0C. The bottles were placed in plastic storage boxes (250x250mm) which contained 400ml of glycerol solution in glass beakers at the same a_w as the treatment condition used to maintain the correct ERH. These boxes were sealed with plastic lids and stored in incubators at the target temperature. Glycerol solutions were changed at monthly intervals. Samples were analysed at 2, 4 and 6 month intervals for mycoflora, using direct and dilution

plating techniques. Dry matter losses (DML) were determined by weighing samples before and after drying in an oven at 115^0C for 24 hours, and calculating dry matter loss using the following formula:-

$$\% \text{ DML} = 100 - \left(\frac{(\text{Dmatday0} - \text{DM of sample}) \times 100}{(\text{DMatday0})}\right)$$

Thus, the mean dry matter of unincubated hydrated samples at day 0 was always 100%.

2. 7 USE OF BIOCIDES TO CONTROL FUNGAL ACTIVITY ON STRAW

2.7.1 In vitro studies on efficacy of biocides over the temperature range 10-30^{0}C on fungal activity

In vitro studies using various biocides concentrated on three main experimental areas, these were:-

1. Control of natural mycoflora using biocides

2. Efficacy of biocide for controlling spore germination

3. Efficacy of biocide against mycelial growth

2.7.2 Control of natural mycoflora of straw using biocides

Direct plating (5 per plate) and serial washings of straw were inoculated onto biocide adjusted media, concentrations of 0.8-100ppm. All experiments were

carried out with three replicates per treatment and incubated for 7 days over the temperature range 10-30^{0}C.

2.7.3 Efficacy of biocides for controlling spore germination

Stock spore suspensions of fungi (see Table 2.4) were obtained from plates by flooding with 20ml of sterile 0.1% agar diluent and the agar surfaces gently rubbed with a surface sterilised glass spreader. The spore concentration was assessed using a haemocytometer and diluted as required. Each inoculant was diluted in 0.1% peptone water to give $3x10^5$ spores ml^{-1}.

The inoculant diluent (0.1ml) was spread-plated onto biocide (Table 2.5) adjusted 2% MEA media (0.8-1000ppm) and 2% MEA. The plates were incubated for up to 7 days at the temperature range 10-30^{0}C and 3mm plugs removed with cork borer and stained with lactophenol/cotton blue, three per treatment. The plugs were examined under the microscope for spore germination after 24 hrs and daily for 7 days. Germination was considered to have occurred when the germ tube was equal to the diameter of the spore.

2.7.4 Biocide efficacy against mycelial growth of fungi

Agar plugs were removed from the growing margins of colonies of each species used (see Table 2.4) using a 3mm diameter cork borer, except for *Rhizomucor*, *Aspergillus* and *Penicillium* species. The plugs were placed in the centre of biocide adjusted agar plates (concentration 0.8-100ppm) and incubated for 7 days.

Table 2.5 The active ingredients of the biocides used in experiments.

Name	Active ingredients
Busan 52 (Buckman laboratories)	32% Potassium N-hydroxymethyl-N-methyldithiocarbamate
Busan 881 (Buckman laboratories)	14.7% Disodium cyanodithioimidocarbonate 20.3% Potassium N-methyldithiocarbamate
Adesol 20 (Coalite Chemicals)	23% Dioclyldimethyl ammonium chloride 20% Ethane diol 5% Ethanol
Lastil 40 (Coalite Chemicals)	33.3% 2,4,6-Trichlorophenol 6.7% Sodium hydroxide

For the *Penicillium*, *Aspergillus* and *Rhizomucor* spp. spore suspensions were first prepared and using an inoculating loop with a diameter of 3mm the solutions were centrally inoculated onto the media. For each species three replicates were prepared. The colonies were measured in two directions at right angles to each other daily for 7 days. Growth rates were determined for each species for all treatments and biocides (see Table 2.5) over the temperature range 10-30^{0}C.

2.7.5 Control of fungal respiratory activity on straw using biocides

To compliment experiments on fungal respiratory activity on straw, the efficacy of certain biocides on controlling respiration were undertaken. The range of biocides tested in this experiment were determined on the basis of the results from in vitro studies using biocides. The biocides used were Busan 881 (8-1000ppm), Lastil 40 (100ppm) and Adesol 20 (100ppm) modified to 0.95 or 0.98 a_w.

Straw was modified to the target a_w by the addition of water (for controls) or biocide and water mixtures to obtain target biocide concentrations. The solutions were sprayed onto the straw with a hand-held atomiser and allowed to equilibrate for 24 hours at 4^{0}C. The respirometer was set up as described in Section 2.5.2 and run for 14 days at 25^{0}C.

2.8 FIELD EXPERIMENTS

2.8.1 Experiment to determine efficacy of various biocides (October 1995-March 1996) (NS2)

All field experiments were carried out at Northern Straw's storage site at Great Heck, North Humberside. Eight bales (500kg on average, dimensions 1.70x2.5m) of the same moisture content (12% wet weight) were used for this experiment (cv.Ribband).

Three biocides were tested based on laboratory studies, these were Busan 881 250 ppm (Buckman Laboratories), Adesol 20 100 ppm (Buckman Laboratories) and Lastil 40 100 ppm (Coalite Chemicals). The control bales were sprayed with water only. Each bale was sprayed with 15L of the appropriate biocides solution on the top surface of the bale using a hand-pump spray, so that the final moisture content achieved was 16% (wet weight basis).

Bales were stacked three bales high, so that each treated bale was exposed to the environment and positioned next to the existing stack. Plate 2.4 showed the stack layout. Samples for microbial analysis were taken at monthly intervals from six areas of the bale. At the end of the 6 month period 1m cores from the top of the bale through to the middle were taken using a coring device (designed by SRI) on a motorised tractor for microbial analysis using direct and dilution plating. Samples were sent to SCRI for cellulose and lignin analysis.

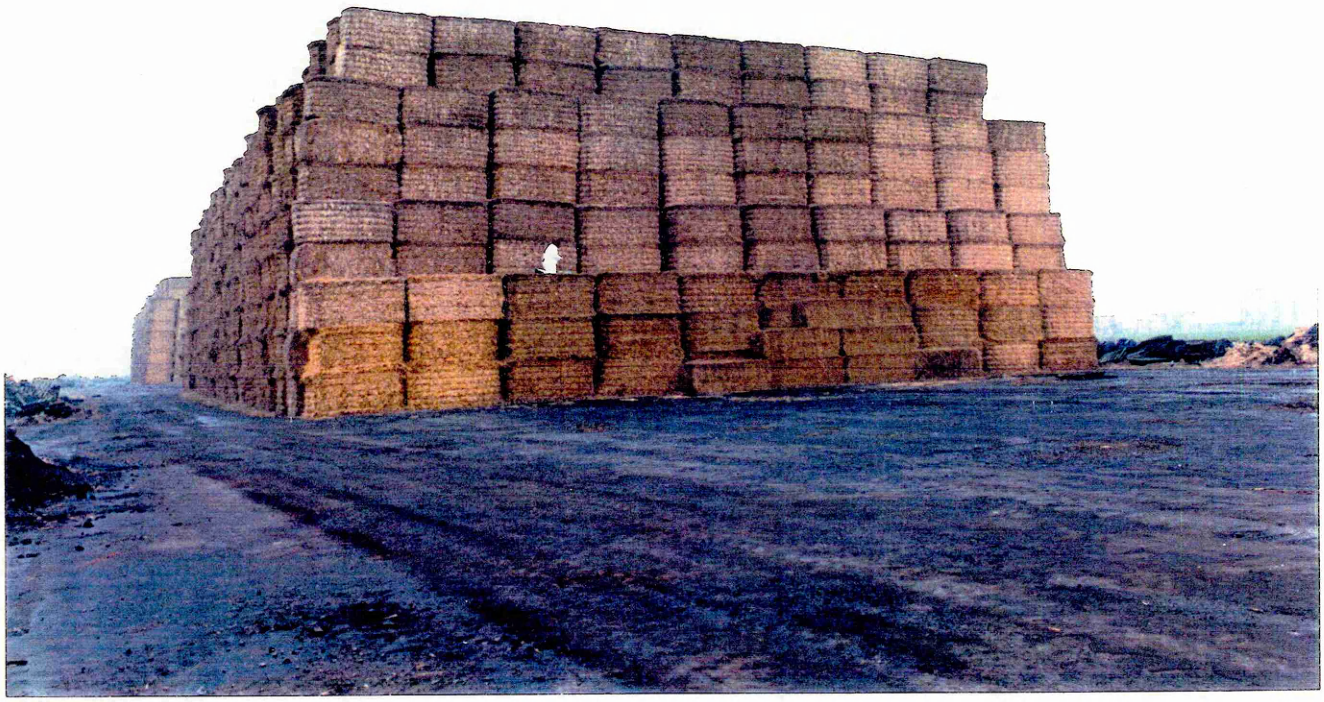


Plate 2.4 The stack layout of the preliminary field experiment.

2.8.2 Efficacy of biocide application on re-baling of straw at various moisture contents (March 1996-August 1996)(NS3)

A more detailed experiment was subsequently carried out to determine the effect of biocide (Lastil 40, 100ppm) treatment of entire bales and surface treatments in bales of different moisture contents (14, 16, 20 and 24% wet weight basis). Initially bales had to be screened for the correct starting moisture content using a portable moisture content probe (Delmhurst). Readings were taken from six positions in the bale to determine the average moisture content. The starting moisture content used was 12% (wet weight basis) this was chosen as the straw is relatively dry and less likely to have microbial deterioration already occurring. In total 79 bales were used for this experiment.

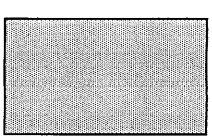
Unless otherwise stated the water/biocide mixture (Lastil 40 100ppm) was sprayed on the straw swath using a spray attachment located on a motorised tractor, then re-baled. Each bale was labelled, weighed and measured at the beginning and end of the experiment (average weight 500kg and 1.70x2.50m), to determine their densities and calculate any dry matter loss resulting from the storage conditions. Table 2.6 and Figure 2.1 showed the treatment procedure and stack plan. Thermocouples were inserted into each of the bales that were to be sampled after 150 days of storage to measure changes in temperature of the stack. The data was recorded onto a logger that could then be transferred to a computer (Plate 2.5).

Table 2.6 Treatment of bales in stack

Moisture content %	Bale number	Treatment
12	1-9	No biocide
12	11-19	Top bale sprayed
16	21-29	No biocide
16	31-39	Biocide
20	41-49	No biocide
20	51-59	Biocide
24	61-69	No biocide
24	71-79	Biocide

Moisture content

	12 %	16 %	20 %	24 %	
	1-3	21-23	41-43	61-63	
	4-6	24-26	44-46	64-66	
	7-9	27-29	47-49	67-69	
	17-19	37-39	57-59	77-79	
	14-16	34-36	54-56	74-76	
	11-13	31-33	51-53	71-73	



Bales surrounding stack

Figure 2.1 Plan of stack layout

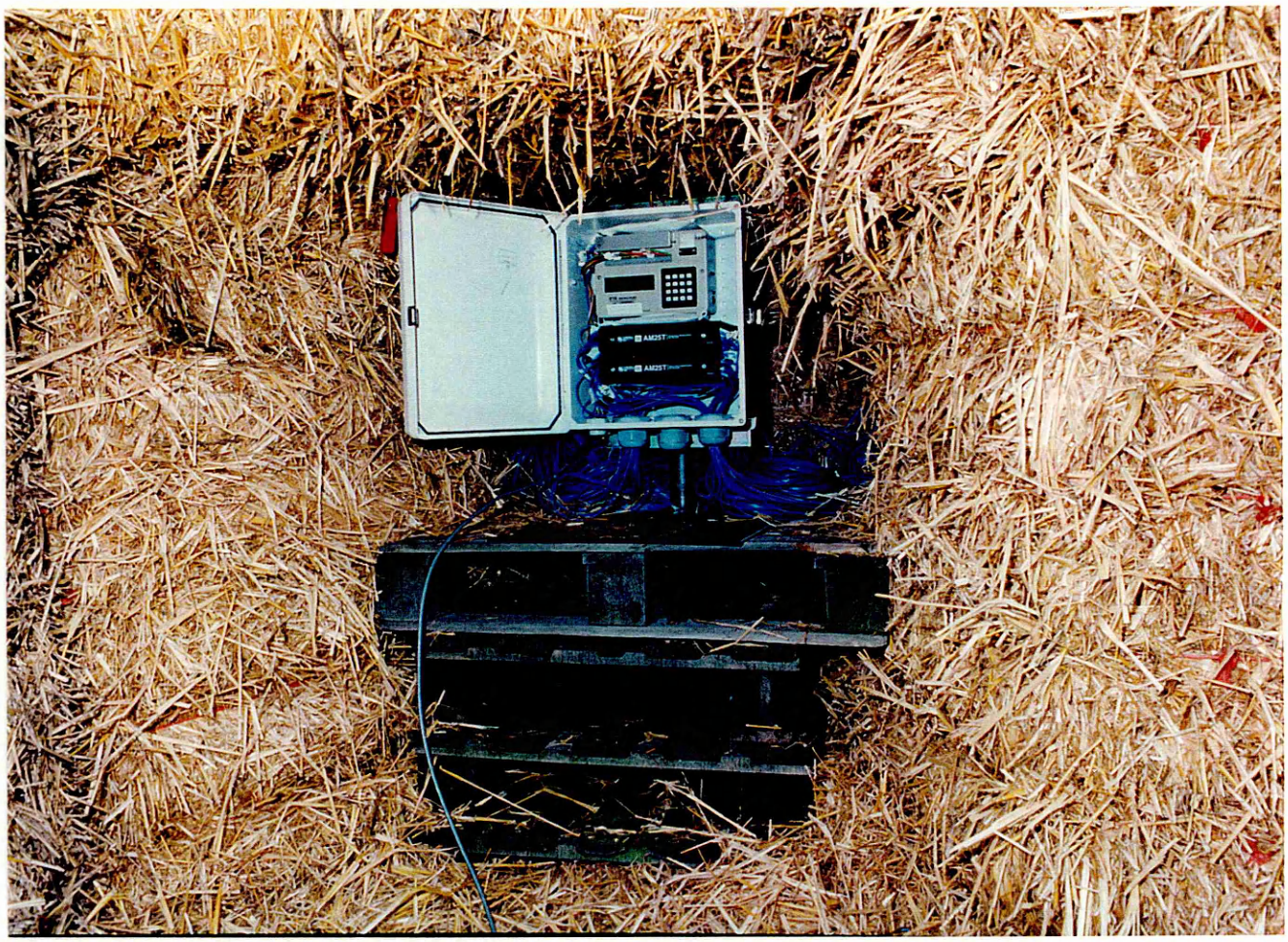


Plate 2.5 Data logger used to record temperature of bales during storage.

The whole stack was built on a layer of non treated bales, so that the stack was four bales high. Throughout the experiment rainfall quantity was measured and recorded daily. Samples were collected at 50, 100 and 150 day intervals, using a coring device (designed by SRI) on a motorised tractor. Samples were analysed for microbial content at Cranfield University, the moisture content at SRI, cellulose and lignin analysis at SCRI and samples for pulping sent to Compak Ltd, Gainsborough.

2.8.3 Investigation of various treatments to improve straw quality during storage (September 1996-February 1997)(NS4)

A more refined experiment was carried out based on the findings from the previous two field experiments. All of the straw baled was from a field containing only the Ribband cultivar (1996) so that the cultivar was not another variable when explaining results. Two moisture contents defined as dry and wet treatments 15% and 23% respectively were used. All treatments were carried out prior to baling. Water or biocide mixture (Lastil 40, 1000ppm) was added to the appropriate bale swath using a spray nozzle attached to a motorised tractor, at either 5L for 15% or 10L for 23%. The same rate of application occurred for both treatments (5L per minute). The swaths were then baled and labelled and the bale size, weight and moisture content determined as in the previous experiment.

Nutri-Shield® was prepared according to manufacturers instructions. Nutri-Shield® ingredients are soy isolate, sodium carbonate, potassium sorbate and polyacrylamide and is manufactured by Nutri-Shield®, Inc. The Nutri-Shield® powder was added gradually to water in the mixing ratio 1lb of powder to 2

gallons of water and mixed thoroughly. The Nutri-Shield® once agitated thoroughly was allowed to stabilise for 4 hours and mixed just prior to application. For Nutri-Shield® treated straw bales, 5L of the solution was sprayed evenly on the top surface of each bale using the spray nozzle at the same application rate as used on the other biocide-treated bales.

Table 2.7 summarises the treatment procedures. Thermocouples were inserted into each of the bales that were to be sampled after 150 days of storage to measure changes in temperature of the stack. The data was recorded onto a logger that could then be transferred to a computer. The whole stack was built on a layer of non treated bales, so that the stack was four bales high. Throughout the experiment rainfall quantity was measured and recorded daily. Samples were collected at 150 day intervals, using a coring device (designed by SRI) on a motorised tractor. Wads of straw in nets (50 cm layer) were laid on to the top surface of bales 41-46. Chopped straw in nets (~10 cm pieces in a 50 cm layer) were laid on to the top surface of bales 51-56. Samples were analysed for microbial content at Cranfield University, the moisture content at SRI, cellulose and lignin analysis at SCRI and samples for pulping sent to Compak Ltd, Gainsborough. All results were statistically analysed.

Table 2.7 Bale treatments

Moisture content %	Bale number	Treatment
15	1-3	Control-water
23	4-6	Control-water
15	11-13	Biocide onto stack
23	14-16	Biocide onto stack
15	21-23	Biocide into straw nets
23	24-26	Biocides into swath
15	31-33	Nutri-Shield®
23	34-36	Nutri-Shield®
15	41-43	Wads of straw in nets
23	44-46	Wads of straw in nets
15	51-53	Chopped straw in nets
23	54-56	Chopped straw in nets

CHAPTER 3

RESULTS

3.1 DETERMINATION OF THE MYCOFLORA OF WHEAT STRAW

Initially an experiment was carried out to determine the length of straw to use for direct plating and isolation frequency of different fungi. Figure 3.1 demonstrates the effect different lengths of wheat straw had on isolation of species numbers. As the length was increased from 1mm to 15mm the number of different species and colony forming units increased (Figure 3.1). It was decided that 10mm lengths would be used in all further experiments because a wide range of species developed and could be enumerated relatively easily, without significant overgrowth. In addition 10mm lengths showed less variation in species numbers than the other lengths.

Table 3.1 showed that the same species were isolated from the two cultivars Beaver and Ribband. However, the dominance of individual species varied between the two cultivars.

The isolation of different fungi from washed and unwashed straw is shown in Figure 3.2 When samples of straw were washed prior to direct plating, a larger isolation frequency was obtained for fungi such as *Aureobasidium pullulans* and *Fusarium culmorum* than from unwashed samples. By contrast, *Cladosporium cladosporioides* was isolated from a greater proportion of unwashed samples. The same trends were observed at all temperatures investigated (10^0C, 15°C, 20^0C, 25°C and 30°C).

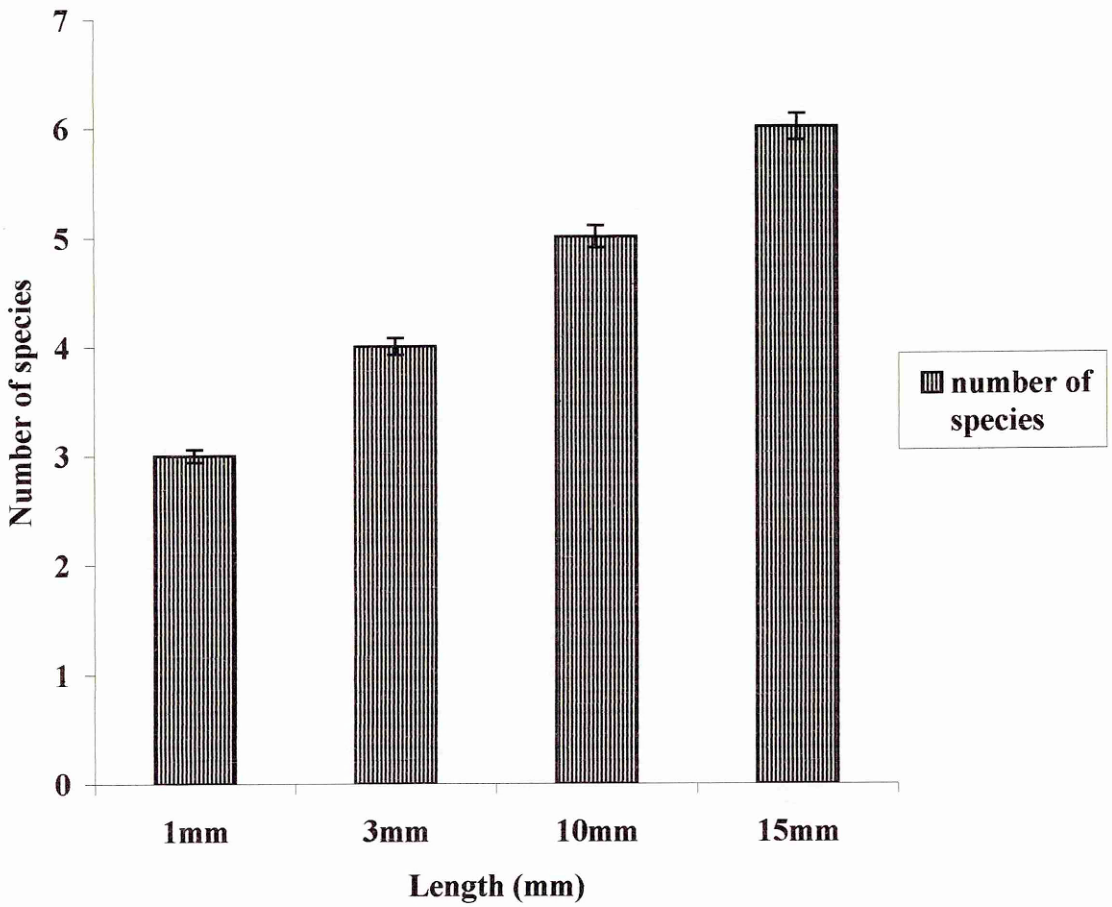


Figure 3.1 The effect different lengths of wheat straw have on growth of species (cv.Ribband).

Table 3.1 Comparison between the total population of fungi (x10^2 CFU g^{-1} straw) versus direct plating (mean percentage colonisation) on MEA media incubated at 25^0C.

	cv. Beaver, 1994		cv. Ribband, 1994	
Species	**x10^2 CFU g^{-1} straw**	**Mean percentage colonisation**	**x10^2 CFU g^{-1} straw**	**Mean percentage colonisation**
E.nigrum	1	33.33	1	80
C.cladosporioides	30	33.33	20	26.66
F.culmorum	30	20	20	20
A.alternata	15	53.33	75	73.33

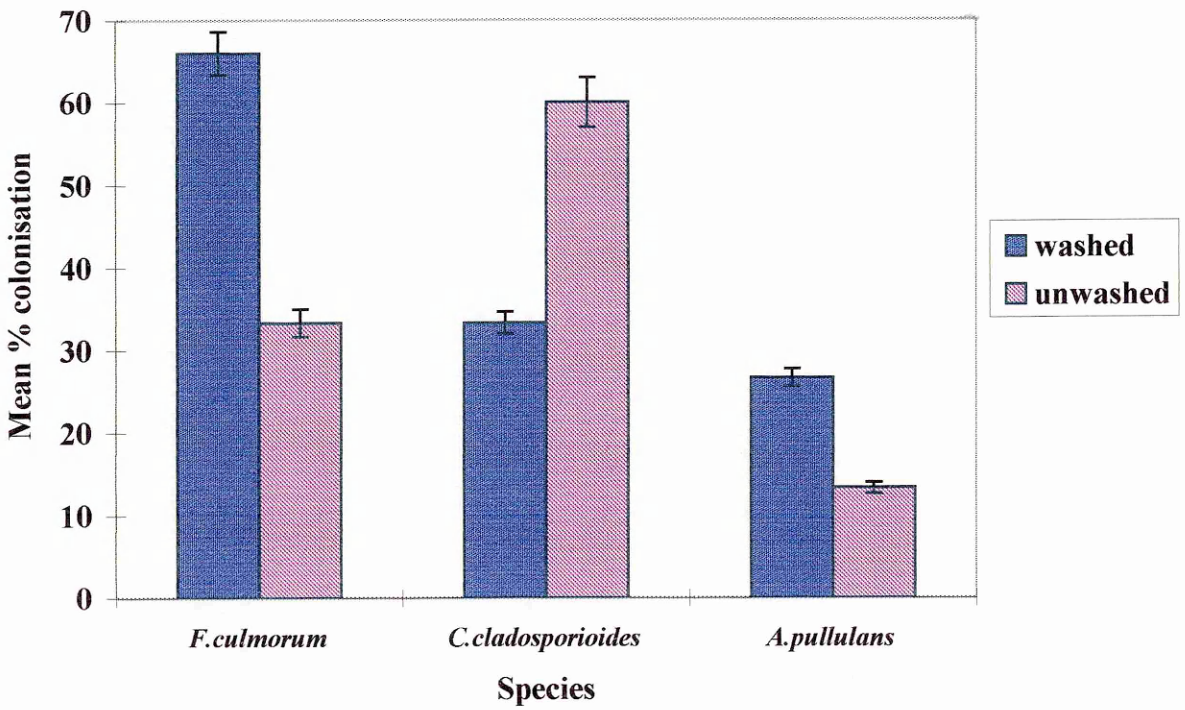


Figure 3.2 The effect washed and unwashed pieces of straw have on fungal isolation when incubated at 10°C on MEA (cv.Ribband).

Figure 3.3 showed that *Cladosporium* and *Fusarium* species were present externally on a greater percentage of straw segments than the internal surface when direct plated. There was low level isolation of *Sporobolomyces roseus* from the external surface and the yeast cells were surprisingly present to a greater extent on the internal structure.

3.1.2 Effect of temperature, a_w and pH on isolation of dominant fungi from straw

Figure 3.4 demonstrates the effect of temperature on isolation of the dominant species on MEA media. As the temperature was increased from 10^0C to 30^0C the number of different species changed. The isolated species varied with temperature, e.g. at 30^0C, *Aspergillus* species were present, whereas at the lower temperatures these species were not isolated at all. Maximum isolation frequencies for *F.culmorum* and *A.pullulans* were at 30^0C and 15-30^0C respectively. This demonstrates that colonisation of straw by different species may be temperature dependant.

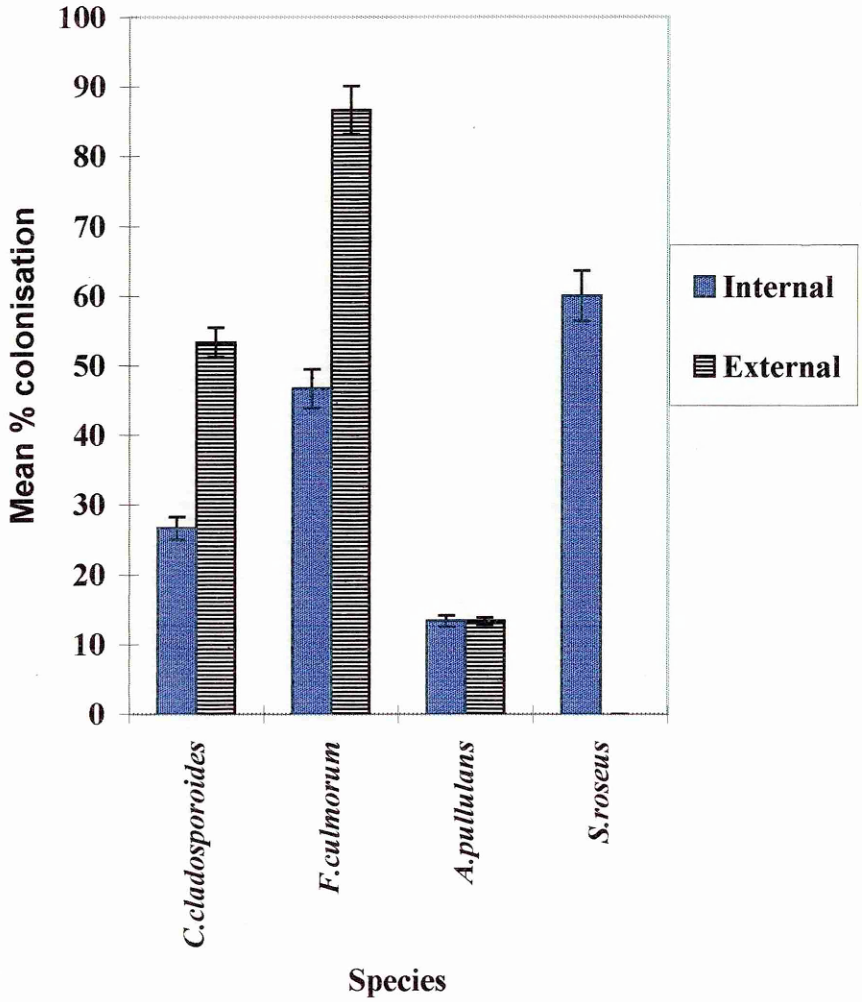


Figure 3.3 The difference between the predominant internal and external mycoflora of wheat straw (cv.Ribband).

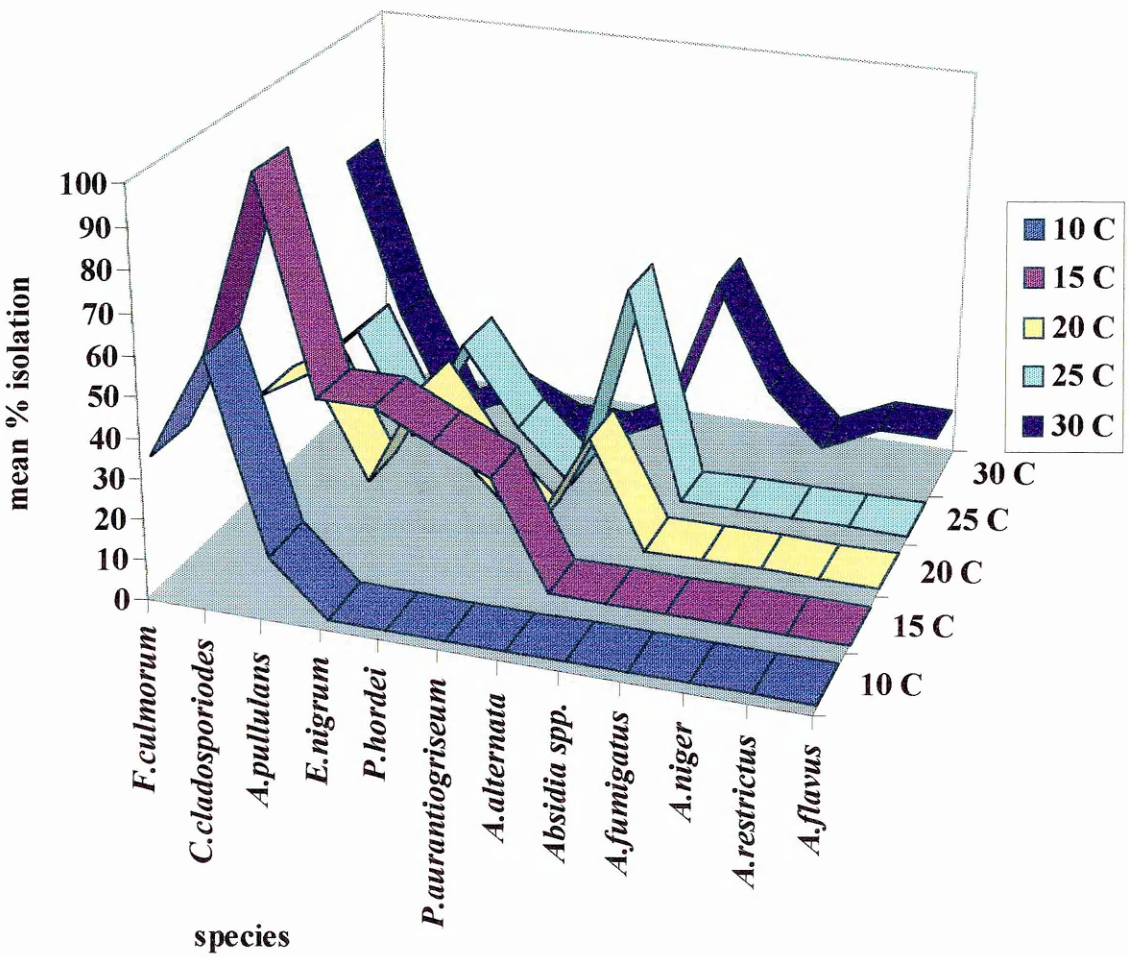


Figure 3.4 The effect of temperature on isolation of fungi from wheat straw after 7 days incubation on MEA (cv.Ribband).

Figure 3.5 demonstrates the effect of plating straw segments on a range of a_w-modified media on genera and species isolated from wheat straw. At 0.75 and 0.80 a_w, *A.flavus* was the dominant species with a smaller proportion of colonisation (13.33% and 20% respectively) by *Eurotium* spp. However, at 0.85 a_w *E.amstelodami* became dominant and *A.flavus* was not isolated. These results show that as the a_w was increased, the range of species and frequency of isolation changed. At 0.95 a_w the greatest range of species were present and the dominant species at 0.75-0.90 a_w were replaced by a different range of fungi. The results show that a_w is an important environmental factor which may influence colonisation and deterioration of straw.

The effect of different medium pH on isolation of fungi from straw at 25^{0}C is shown in Figure 3.6 The types of species varied with pH; at 4.6 and 4.8 *P.aurantiogriseum* was present, whereas it was not isolated at the other pH levels examined. However, *A.alternata* was present at all pH levels tested (4.4-6.4).

The mean percentage isolation varied markedly with pH. For example, the mycelial yeast *A.pullulans* was isolated from 40% of straw at pH 6.2 but only 20% at pH 6.4. Therefore the degree of isolation and type of species colonising the straw may also be pH dependent. The experiments were repeated at 10^{0}C, 15^{0}C, 20^{0}C and 30^{0}C, with similar trends being observed. The exception was that at 30^{0}C there were more thermotolerant species, such as *A.fumigatus*, *Absidia* spp. and *A.flavus* present. The overall mean isolation levels increased from 10^{0}C to 30^{0}C, with very little growth occurring at 10^{0}C, and the maximum at 25^{0}C.

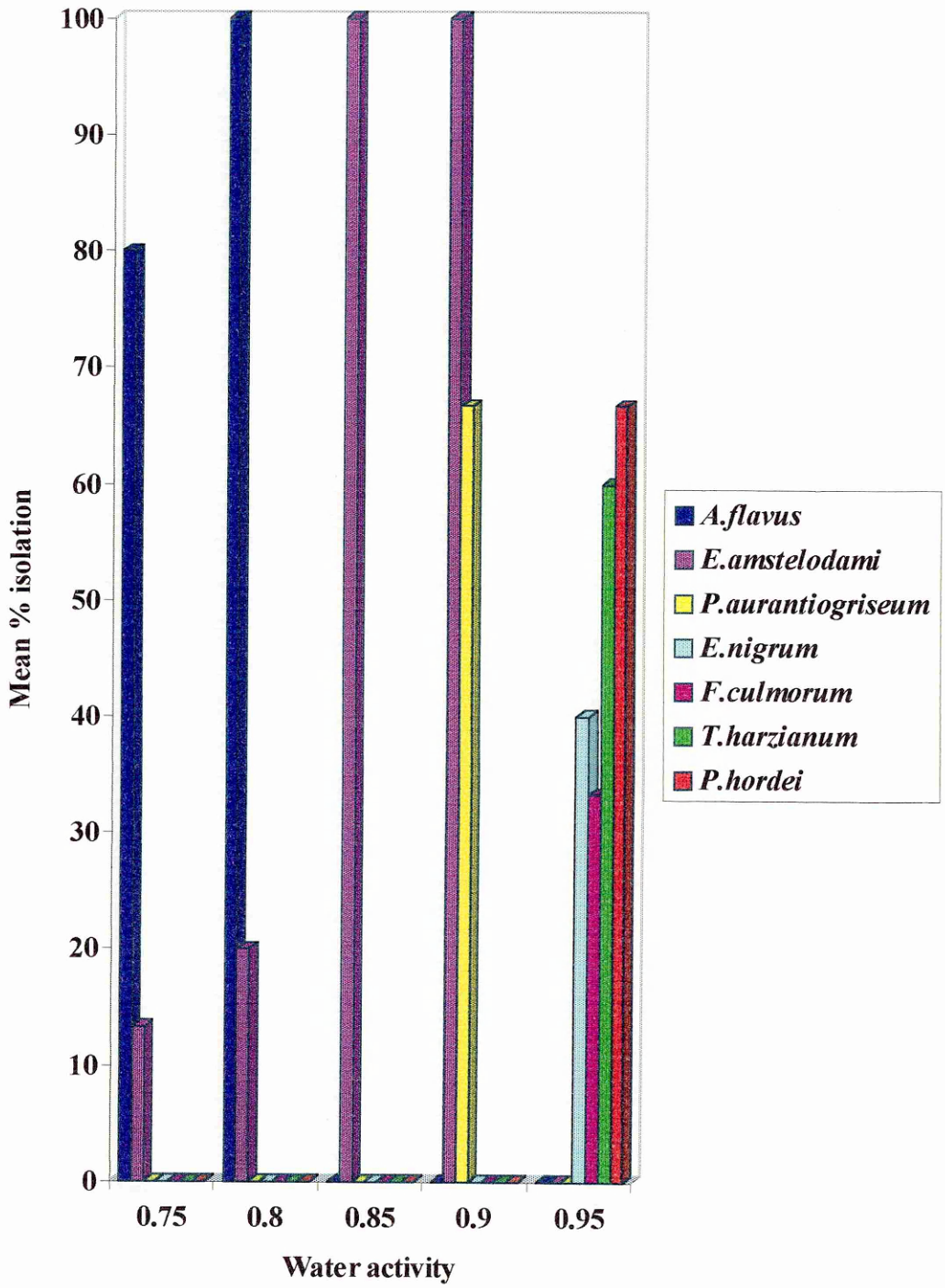


Figure 3.5 The effect of a range of a_w levels on fungal isolation from straw segments at 25^{0}C (cv.Ribband).

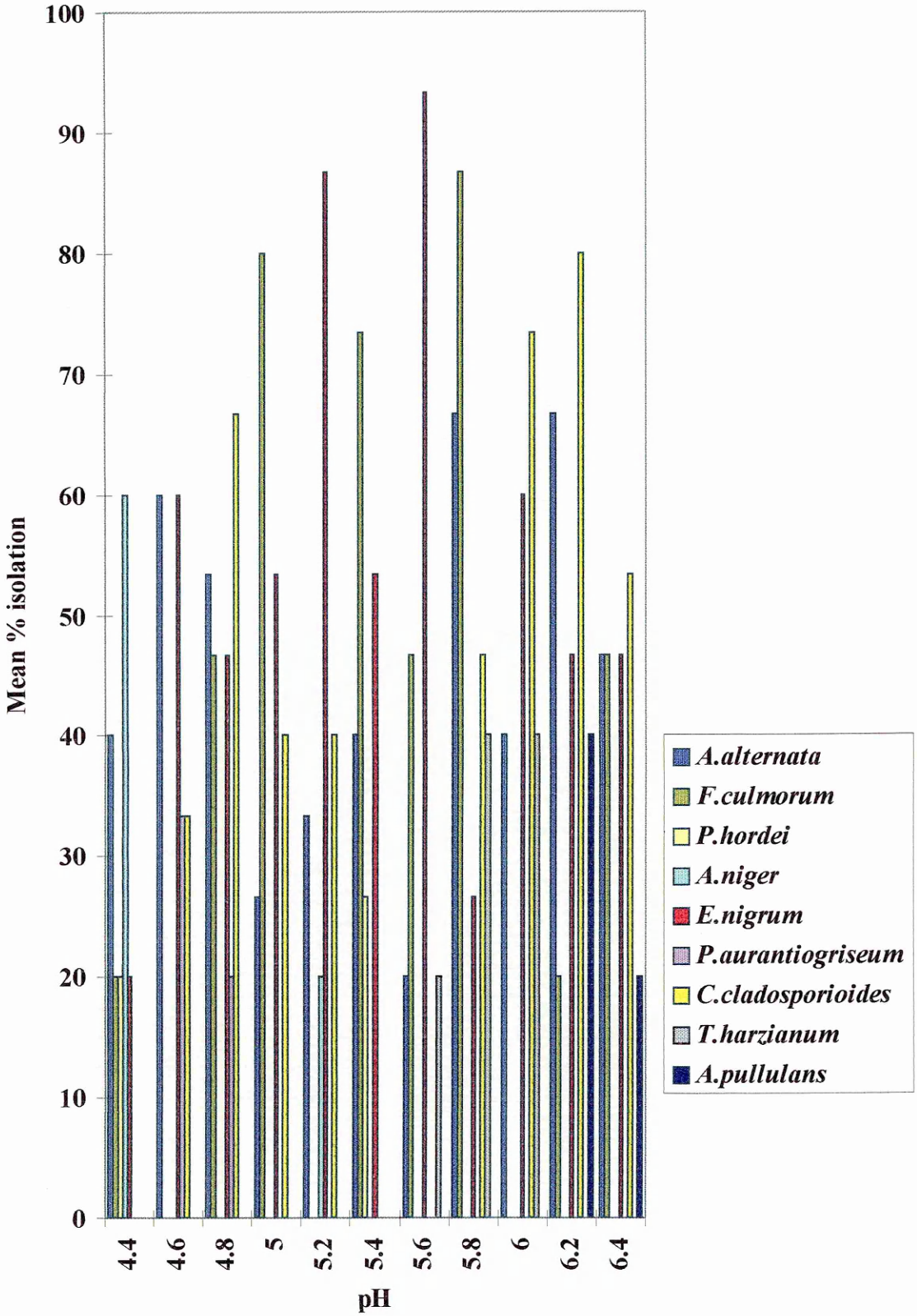


Figure 3.6 The effect of a range of pH on fungal isolation from straw segments at 25^{0}C (cv.Ribband).

3.1.3 In vitro studies on effect of temperature, a_w and pH on growth of dominant species

Table 3.2 showed the effect of temperature and a_w on the radial growth of dominant fungi on 0.95 a_w-adjusted media incubated at 25^0C. The species *F.culmorum*, *R.pusillus* and *T.harzianum* achieved maximum growth of 84mm over the 7 day period indicating that the environmental conditions were conducive for these species. All species grew under these conditions.

Table 3.3 demonstrates the effect of temperature, a_w and pH on growth of the dominant fungi. For each fungus as the a_w increased, the rate of radial growth increased slightly. The same trend was seen for the other temperatures investigated.

3.1.4 Determination of moisture content, a_w and adsorption isotherms

Table 3.4 showed the relationship between a_w and moisture content of straw. This showed that as temperature increased the moisture content, at a given a_w decreased. Whereas moisture content increased with a_w, this trend was seen for all of the temperatures. The relationship between known amounts of added water and the a_w of wheat straw cv. Beaver, 1994, is shown in Figure 3.7. This showed that as the a_w increased the volume of water to be added increased at a given temperature. As the temperature decreased from 30^0C to 10^0C the volume of water to be added increased at any set a_w. Consequently adsorption of water at 30^0C was greater than at the lower temperatures, therefore less water needed to be added to achieve the same a_w due to the hysterisis effect.

Table 3.2 The radial growth of fungi inoculated onto 0.95 a_w-adjusted media and incubated at 25^{0}C

	Radial Growth (mm)						
Species	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
A.alternata	11	19	26	33	37	40	45
A.pullulans	9	13	22	26	28	34	37
C.cladosporioides	8	9	10	11	11	13	14
E.amstelodami	3	4	7	10	12	13	15
E.nigrum	9.5	18	25	34	40	46	52
F.culmorum	13	33	53	77	84	84	84
R.pusillus	9	19	33	59	77	84	84
P.aurantiogriseum	4	8	15	21	26	31	37
P.hordei	4	5	9	10	13	16	20
S.roseus	7	9	10	11	12	13	13
T. harzianum	15	33	57	84	84	84	84

Table 3.3 The effect of pH (4.4), a_w (0.90, 0.95) and temperature (10^0C) interactions on the growth rate of dominant fungi

	Growth rate (mm day^{-1})	
Species	**0.90 a_w**	**0.95 a_w**
A.alternata	1.3	1.7
A.pullulans	2.5	3
C.cladosporioides	1.3	1.8
F.culmorum	4.0	4.5

Table 3.4 Mean moisture contents at different a_w at various temperatures for wheat straw, cv. Beaver, 1994.

a_w	Moisture Content (percentage wet basis)			
	10^{0}C	**15^{0}C**	**25^{0}C**	**30^{0}C**
0.75	13	11.65	10.89	9.9
0.80	13.70	12.33	11.45	10
0.85	14.28	14.79	12.19	10.43
0.90	17.08	16.50	13	10.61
0.95	17.30	17.27	14.42	13.59

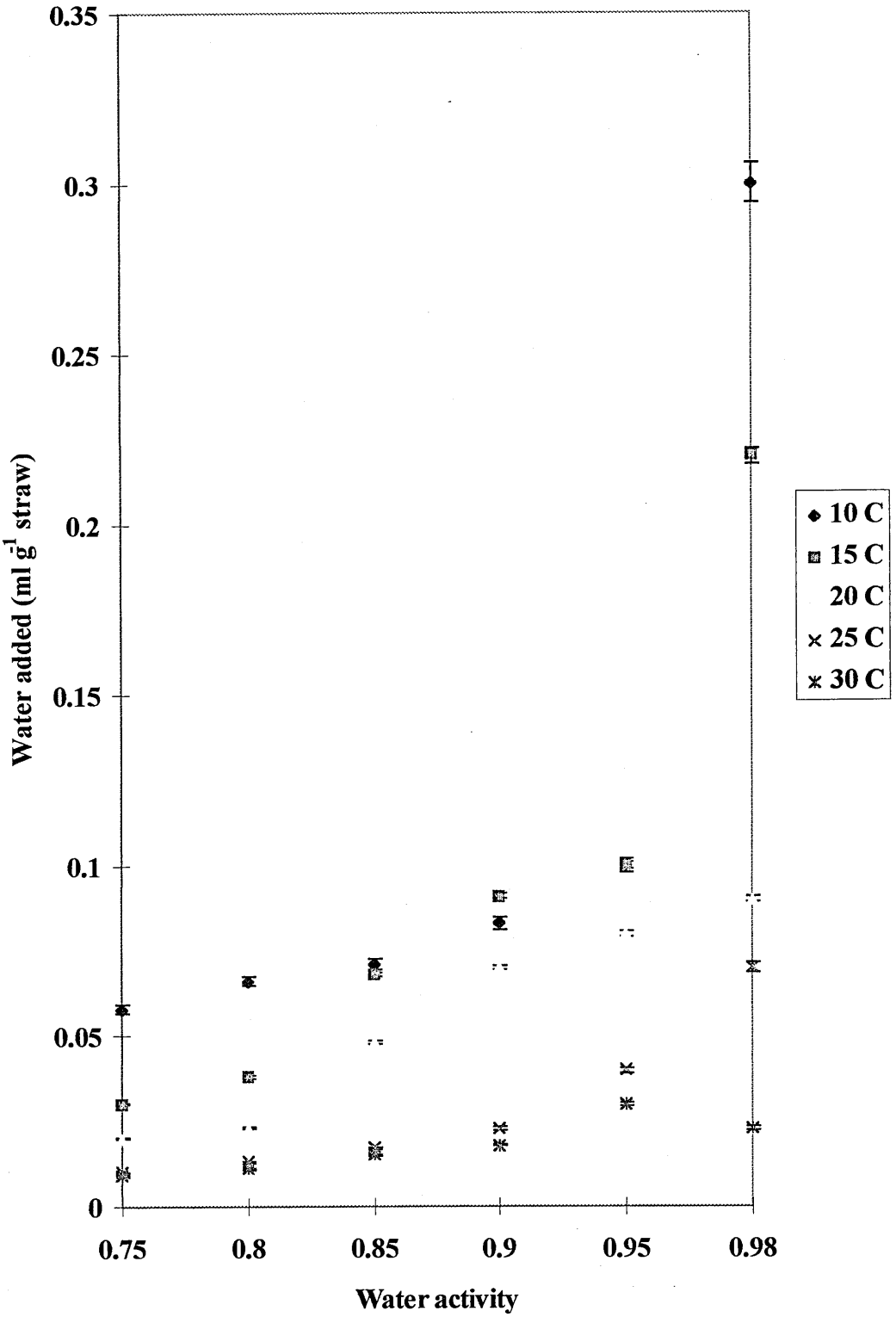


Figure 3.7 Water adsorption isotherms for wheat straw (cv.Ribband).

3.2 EFFECT OF ENVIRONMENTAL FACTORS ON FUNGAL RESPIRATION ON WHEAT STRAW

3.2.1 Temperature x a_w interactions on fungal respiration on straw

The temperatures investigated were 10^0C, 15^0C, 20^0C, 25^0C and 30^0C over the a_w range 0.75-0.98. The data all represent means of three replicates, with the experiments repeated once.

Figure 3.8 showed the temporal changes in the respiratory activity of natural fungal populations at different steady-state temperatures. This demonstrates that there was a steady increase in respiratory activity as the temperature was increased. At the highest temperature investigated (30^0C) there was almost a linear increase, whereas at the lowest temperature (10^0C) a lag time occurred prior to fungal respiratory activity. This showed that fungal colonisation of the straw was significantly (P <0.05) influenced by temperature alone and may be an important environmental factor affecting fungal activity.

Figure 3.9 demonstrates that the same trends occurred in relation to a_w. At high a_w (0.95) the oxygen consumption values for each temperature was almost 50% greater than at the corresponding temperatures at low a_w (0.75). This showed that fungal activity and colonisation of wheat straw was significantly (P <0.05) influenced by a_w alone.

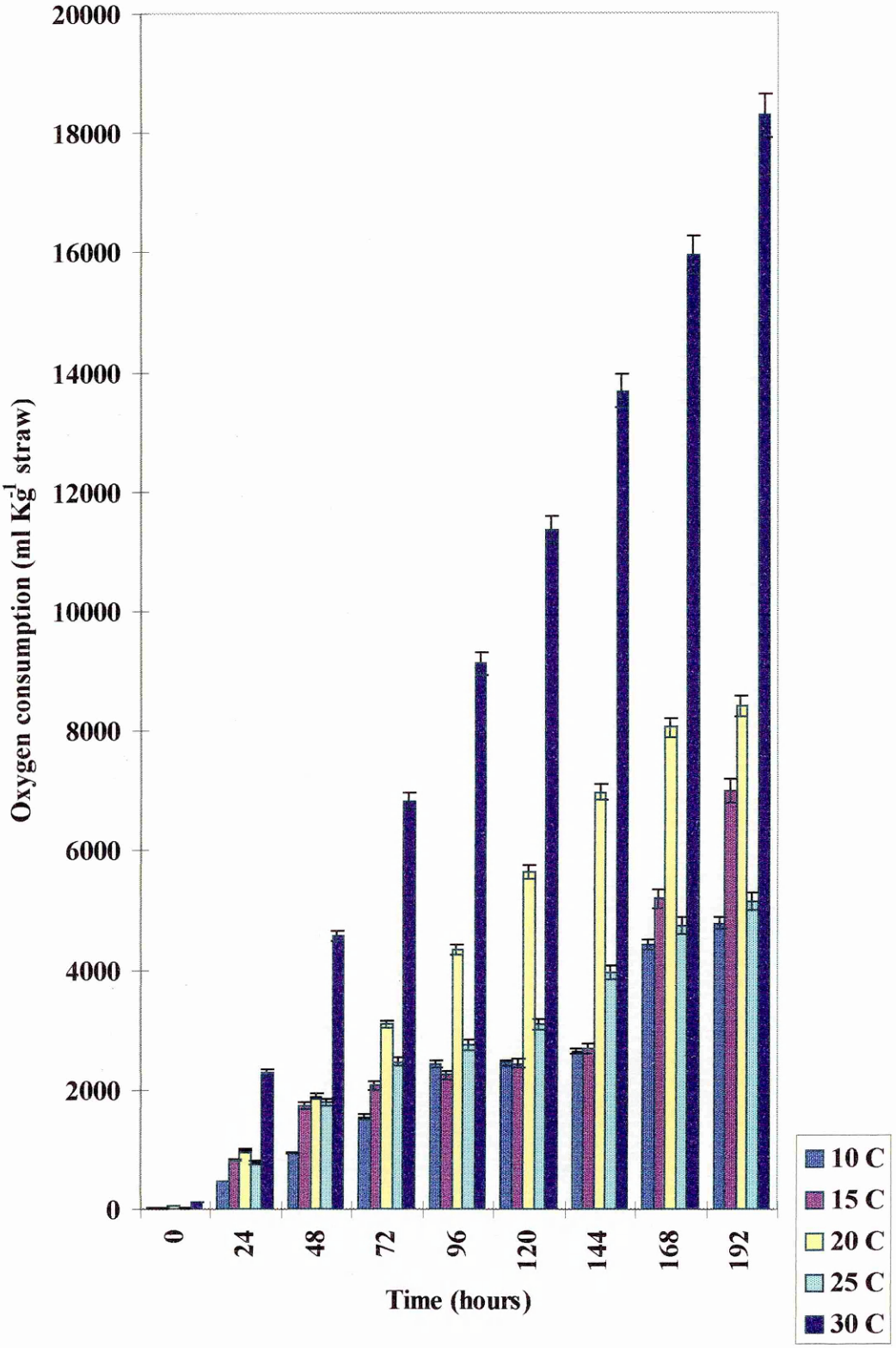


Figure 3.8 The effect of temperature on fungal respiration on a_w-adjusted unsterile straw (0.75) (cv.Ribband).

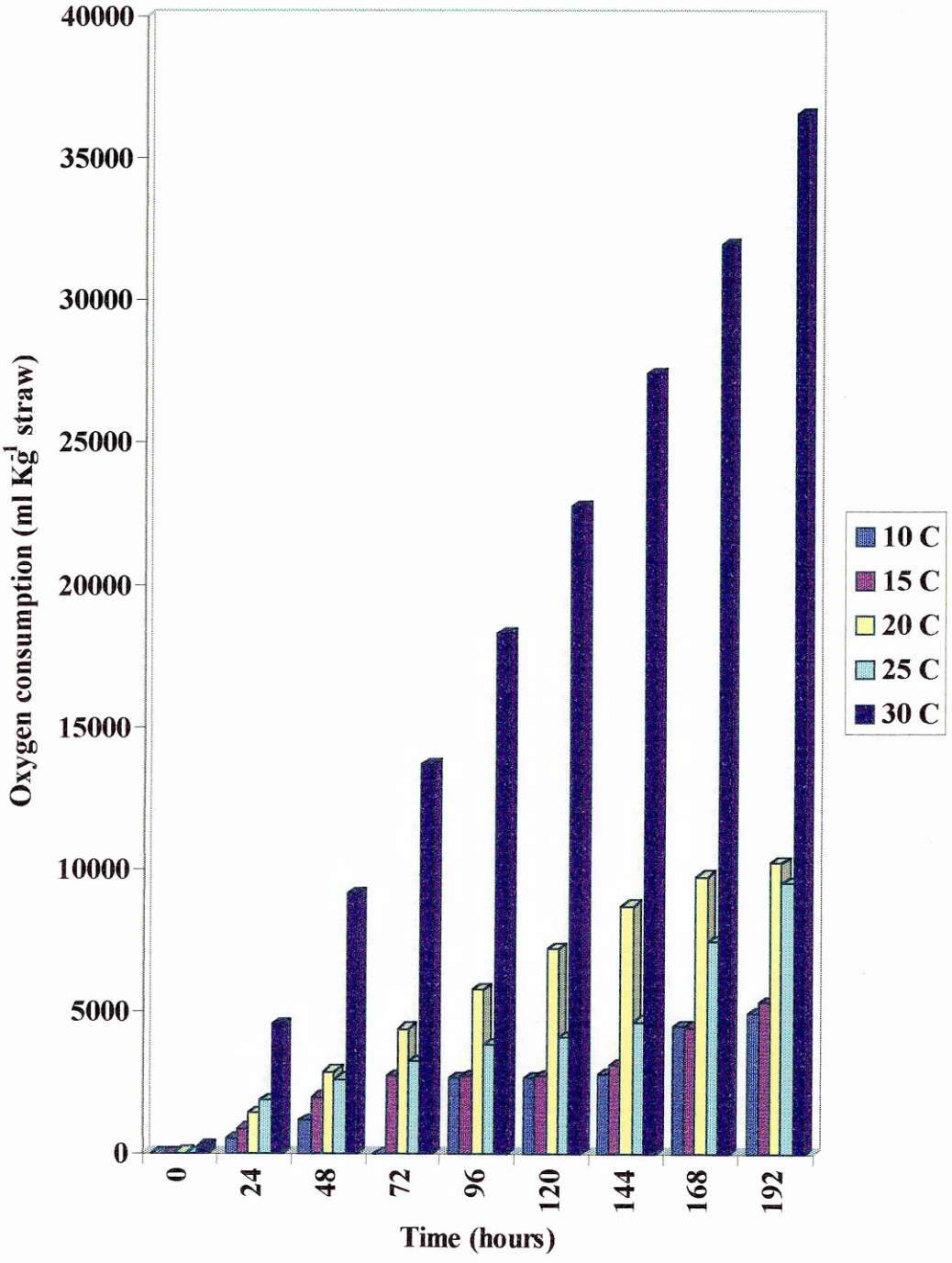


Figure 3.9 The temporal increase in accumulated respiratory activity of fungi on wheat straw at different temperatures at 0.95 a_w (cv.Ribband).

Figure 3.10 showed there was a steady increase in respiratory activity as a_w was increased over a period of time at 25^{0}C. The respiratory activity at 0.98 a_w was almost three times that at 0.75 a_w. At all of the a_w levels up to 24 hours there was little respiratory activity and a lag phase could be clearly seen.

Figure 3.11 showed that as temperature was modified from 10-30^{0}C at different steady-state water activity there was a change in respiratory activity of fungi on the straw. Generally the respiratory activity increased with temperature and water content. This showed that temperature x a_w interactions are important factors influencing fungal colonisation and respiration.

3.2.2 Enumeration of fungal species from respiration studies

At the end of each respirometer experiment sub-samples of straw were removed from respirometer bottles and the microbial population enumerated. Fungal populations varied with varying a_w. *E.amstelodami* was isolated over the widest a_w range (0.75-0.90) when compared to the other species (Table 3.5).

Table 3.6 showed at high water availability (0.98 a_w) the *Aspergillus* species; *A.flavus*, *A.niger*, *A.fumigatus* and *Absidia* species, were only present at the highest temperature tested, 30^{0}C, showing that they were thermotolerant. *F.culmorum* was the only species present over the whole temperature range, which indicates a wide adaptability to the environmental conditions. Three species (*F.culmorum*, *A.pullulans* and *C.cladosporioides*) were isolated at the lowest temperature of 10^{0}C. Surprisingly the optimum colonisation of *C.cladosporioides* was at 10^{0}C and not at 25^{0}C.

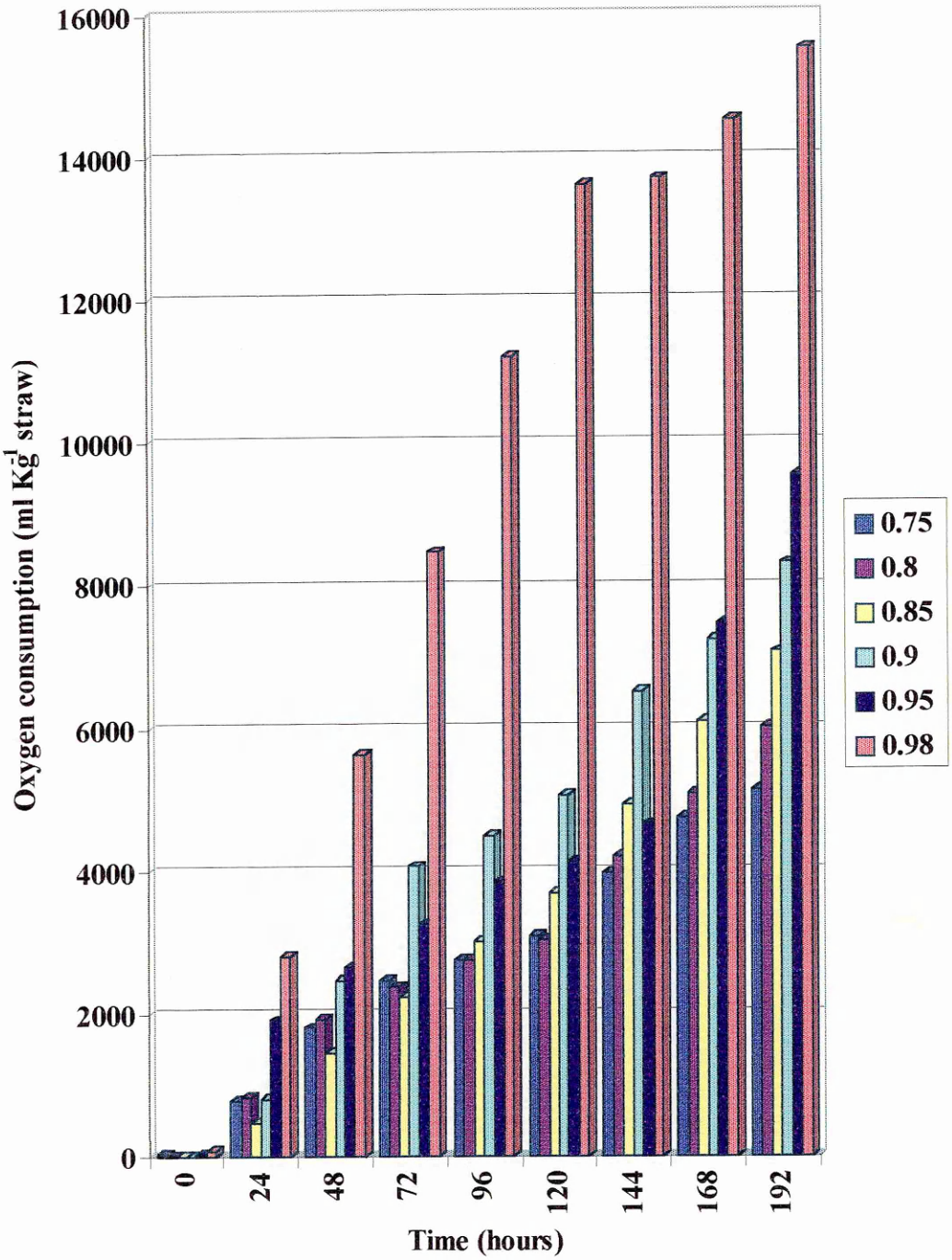


Figure 3.10 The effect of a range of a_w on fungal respiration at 25^0C on straw (cv.Ribband).

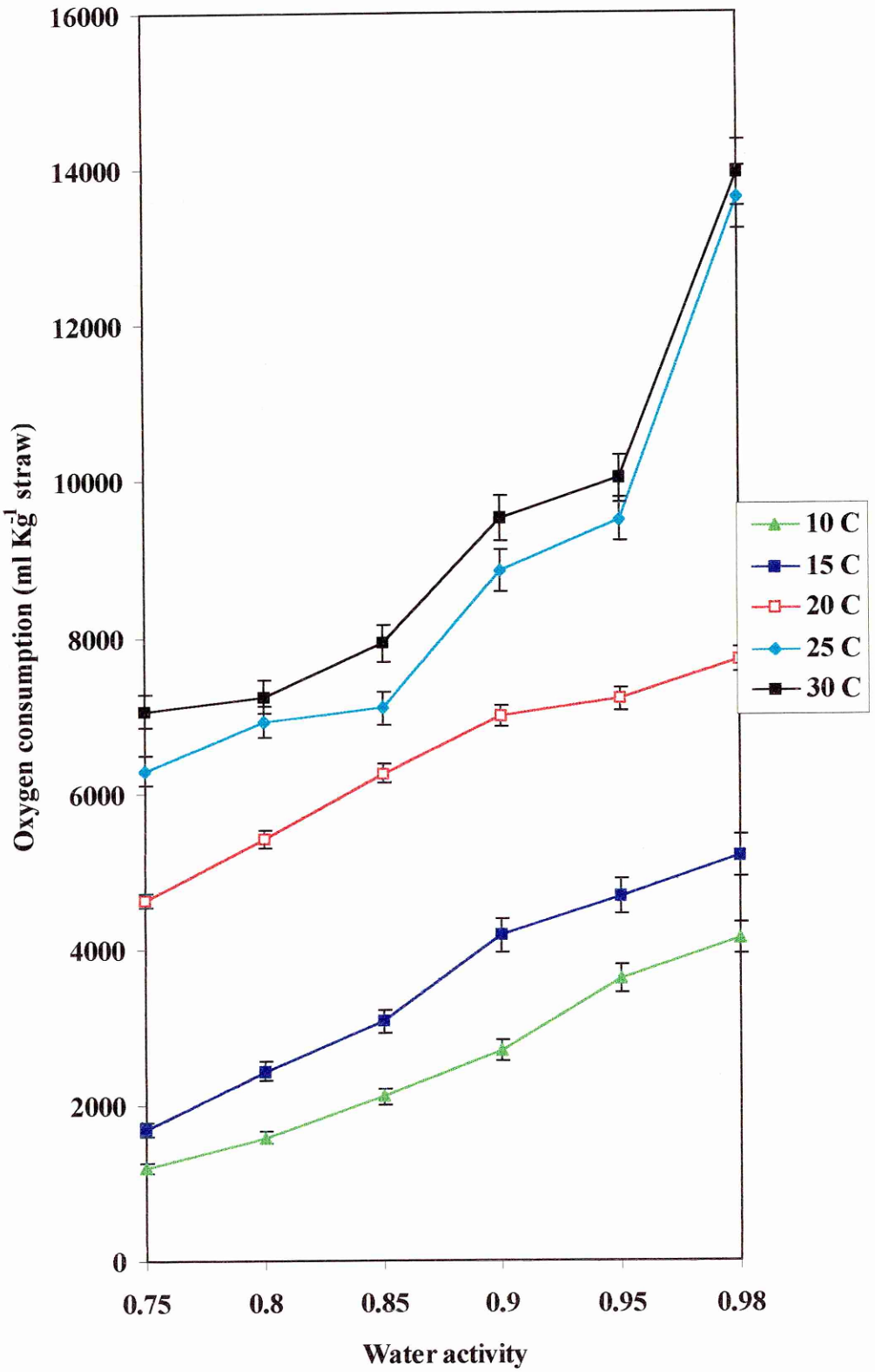


Figure 3.11 The effect of water activity and temperature on the respiratory activity of fungi on naturally contaminated straw (cv.Ribband).

Table 3.5 The mean percentage dominant species isolated at different a_w from straw at 25^{0}C after 14 day experimental period.

	Mean percentage colonisation					
Species	**0.75**	**0.80**	**0.85**	**0.90**	**0.95**	**0.98**
A.flavus	0	83	0	0	0	0
E.amstelodami	50	17	100	60	0	0
P.hordei	50	0	0	40	67	0
E.nigrum	0	0	0	0	16	20
F.culmorum	0	0	0	0	16	20
A.alternata	0	0	0	0	0	60

Table 3.6 Fungal species isolated from straw after 14 day respiratory experiment at 0.98 a_w and 10-30^{0}C.

	Mean Percentage Colonisation				
Species	**10^{0}C**	**15^{0}C**	**20^{0}C**	**25^{0}C**	**30^{0}C**
A.flavus	0	0	0	0	6.66
E.amstelodami	0	0	0	0	0
P.hordei	0	13	0.80	0	0
E.nigrum	0	15	21	20	0.30
F.culmorum	31.30	20	12.50	20	32.30
A.pullulans	12.50	15	0.40	0	0
C.cladosporioides	56.30	36	21	20	13
A.niger	0	0	0	0	0.30
A.fumigatus	0	0	0	0	0.90
Absidia spp.	0	0	0	0	22.50
A.alternata	0	0	33.33	60	0.30

3.2.3 Dry matter loss determination

The formula used to calculate % DML from oxygen consumption data, assuming RQ = 1.0 was as follows:-

% DML = R x S x T x U

Where: R = 1 O_2 or CO_2/Kg dry straw

S = 44/22.44 (conversion from volume CO_2 to mass O_2)

T= 0.682 (relates to mass CO_2 produced to mass carbohydrate utilised; 1g for 0.682g respectively (Rees, 1982))

U = 100/1000 (0.1) to convert weight of DML to percentage DML

Table 3.7 showed there was a correlation between temperature and a_w interactions in relation to DML at 25^0C and 30^0C. As a_w was increased the calculated DML also increased. Under high a_w conditions (0.90-0.98) as the temperature increased from 10-30^0C the amount of DML increased; however, this trend was not seen for the other a_w levels tested. Except for straw adjusted to 0.75 a_w the maximum dry matter loss occurred when straw was stored at 30^0C over the 14 day experimental period. This correlated with changes in lignin content observed by SCRI (results not shown).

Table 3.7 The effect of a_w and temperature on calculated dry matter loss (DML) in wheat straw after 14 days incubation.

a_w	Mean DML (%)				
	Temperature (^{0}C)				
	10	**15**	**20**	**25**	**30**
0.75	0.35	0.49	0.60	0.32	0.33
0.80	0.18	0.50	0.61	0.39	0.66
0.85	0.19	0.36	0.66	0.49	1.47
0.90	0.31	0.44	0.48	0.56	2.59
0.95	0.33	0.38	0.73	0.74	3.39
0.98	0.35	0.36	0.80	0.81	3.40

At the end of each experiment analysis of lignin and non-cellulosic polysaccharide (NCP) contents of the straw samples were assessed at SCRI. Only the results of the lignin determinations and the DRIFT spectra are presented. The lignin results are the mean of three replicates and each replicate was analysed in triplicate.

Figure 3.12 showed that at 10^0C there was no significant change (P >0.06) in lignin content irrespective of a_w, while, at 15^0C, there appeared to be a slight reduction at low levels of a_w which increased as the a_w increased. The greatest increase was observed on storage at 20^0C when the apparent lignin content rose with increasing a_w and, once 0.90 a_w had been reached, remained at 140 % of the starting value. This indicated significant loss of the carbohydrate components, assuming no loss of true lignin. The results for 25^0C and 30^0C were less clear. At the lower a_w levels there was an increase in apparent lignin content but they were not as high as at 20^0C

The DRIFT spectra shown in Figure 3.13 confirmed that degradation of carbohydrate was occurring. This was particularly evident when a comparison was made of the absorbances in the region 1050-1150 cm^{-1} . Absorbances which were discrete in the untreated straw were either lost or become less discrete in the stored sample.

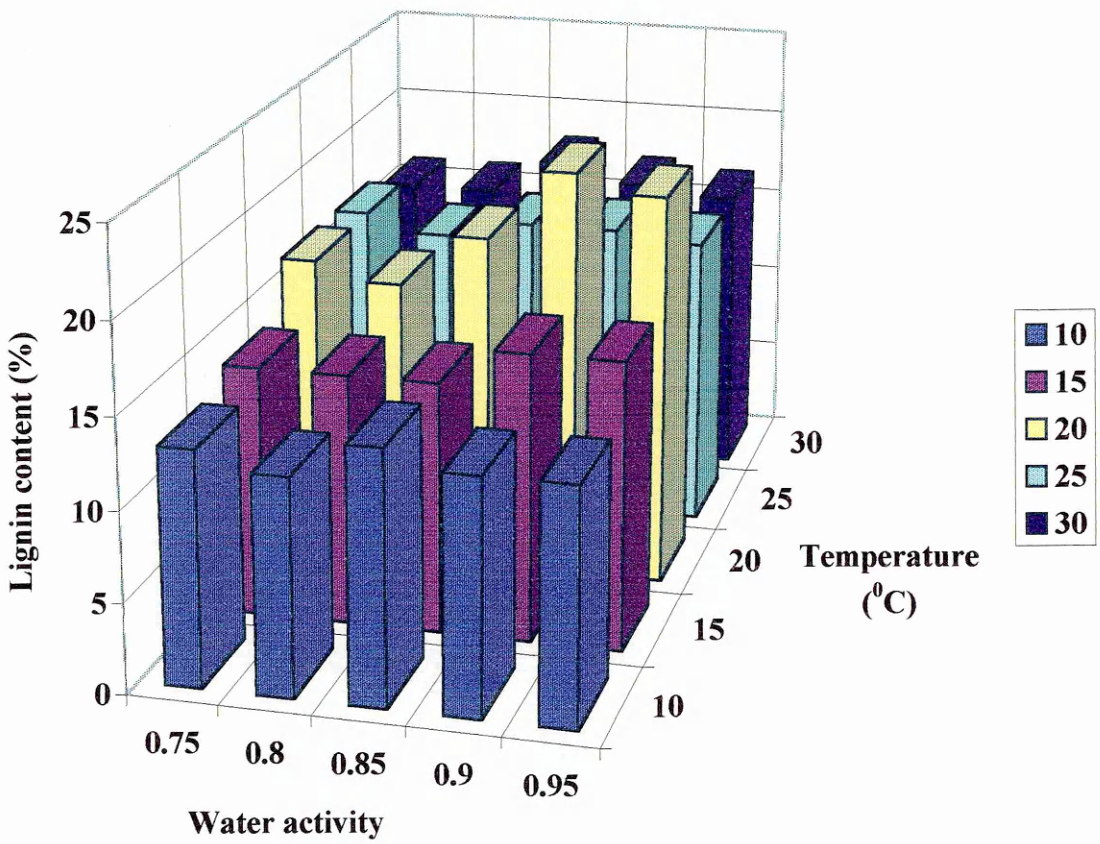


Figure 3.12 Changes in lignin content after storage at different water activities.

(Mean lignin content of control straw (cv.Ribband) was 13.8 %.)

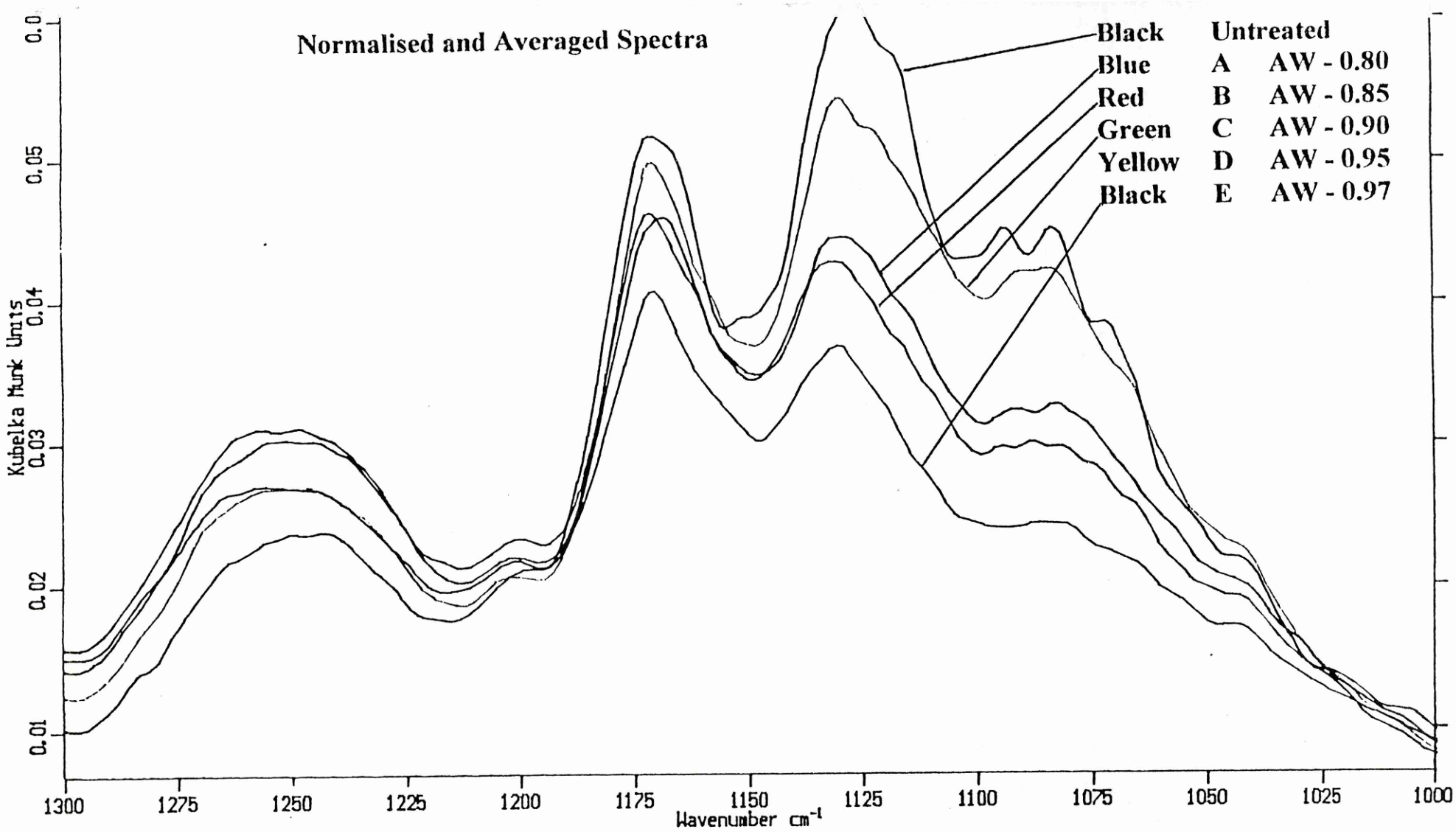


Figure 3.13 DRIFT spectra of wheat straw stored at 15^{0}C at different a_w levels.

3.2.4 Measurement of respiratory activity of component fungal species on straw

The total respiratory activity of fungal species on straw in the controlled environment studies was recorded as cumulative oxygen consumption. The effect of individual fungal species on respiration is an important aspect that needs to be considered. The activity of each species in causing deterioration of the straw was evaluated so that the most important species could be identified.

Figure 3.14 showed that *F.culmorum* had the greatest temporal cumulative oxygen consumption when grown in pure culture on sterile straw. Under the same conditions *C.cladosporioides* had the lowest oxygen consumption. This demonstrated that each species had relatively different respiration rates and that interactions with other species may influence the overall respiratory activity.

Figure 3.15 clearly demonstrated the relationship between temperature and fungal respiration. Each of the species increased their oxygen consumption at 25^0C when compared to that at 10^0C. *F.culmorum* nearly doubled its oxygen consumption at 25^0C when compared to storage at 10^0C. This emphasises that temperature influences the respiration of individual fungal species and is an important environmental factor, which influences deterioration of straw. Table 3.8 showed that for each of the species studied, as the a_w increased the cumulative oxygen consumption increased. Table 3.9 showed that each individual species cause different DML on straw. Generally, as a_w increased the DML increased. The same trend was seen at the other temperatures investigated. *F.culmorum* caused the greatest DML of straw when compared to the other species.

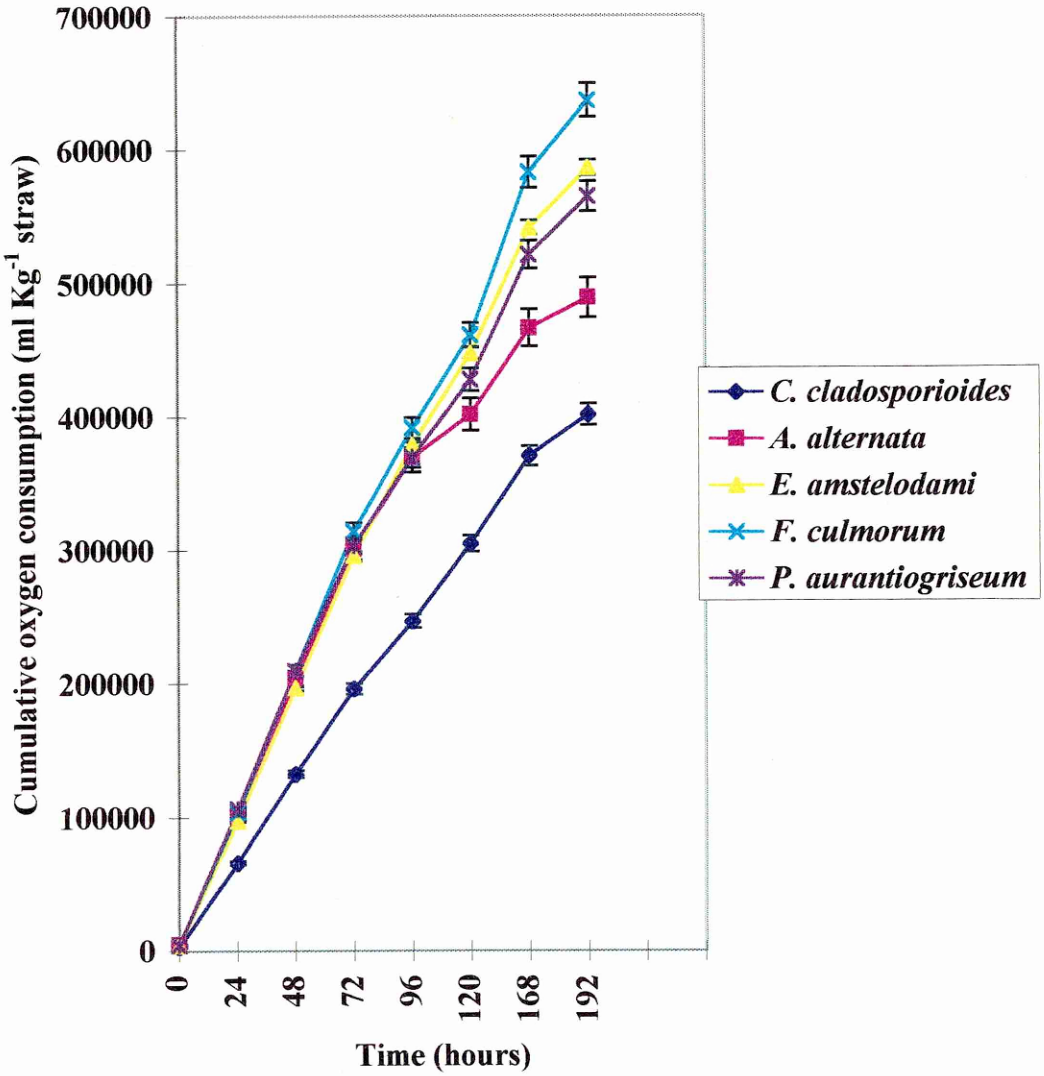


Figure 3.14 The effect of a_w (0.85) and temperature (10^0C) on respiration of individual fungal species.

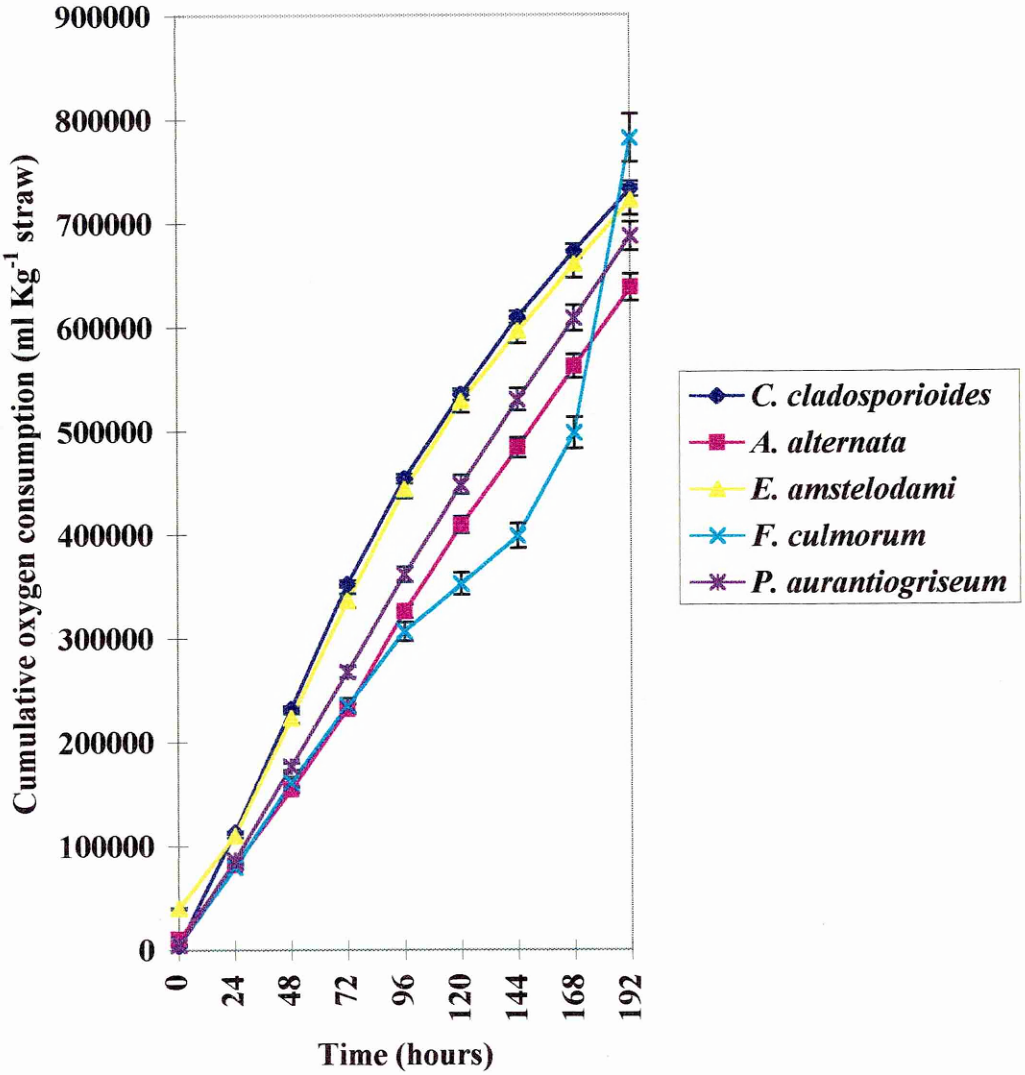


Figure 3.15 The effect of a_w (0.85) and temperature (25^{0}C) on respiration of individual fungal species.

Table 3.8 The cumulative oxygen consumption of individual species on wheat straw after 14 days of storage at 25^0C under various a_w conditions

Species	Mean cumulative oxygen consumption (ml Kg^{-1} straw)		
	a_w		
	0.85	**0.95**	**0.98**
C.cladosporioides	1210760	1557721.50	1610025
A.alternata	1001753.50	1448095.50	1450045
E.amstelodami	1214519.50	1334042.50	1385000
F.culmorum	1773968	1577171.50	1665000
P.aurantiogriseum	1134066.50	1455806.50	1551000

Table 3.9 The effect of individual species on DML in wheat straw after 14 days incubation at 25^0C.

Species	Mean DML (%)		
	a_w		
	0.85	**0.95**	**0.98**
C.cladosporioides	8.09	10.41	10.76
A.alternata	6.69	9.68	9.69
E.amstelodami.	8.11	8.91	9.26
F.culmorum	11.85	10.54	11.93
P.aurantiogriseum	7.75	9.73	10.37

3.3 EFFECT OF a_w AND TEMPERATURE ON FUNGAL SUCCESSION ON STRAW

Storage experiments over periods of 2, 4 and 6 months were undertaken to determine the succession of fungi on straw and the effect of interaction of a_w and temperature on dry matter losses.

The general trend was shown in Table 3.10 Total fungal populations at all temperatures during the storage period were increased and there was an increase in the total CFU g^{-1} straw, regardless of a_w.

Table 3.11 shows that *P.aurantiogriseum* was the dominant species at all a_w levels during storage at 10^0C for 2, 4 and 6 months. *S.roseus* was isolated from 20% of the straw segments after 2 months at 0.85 a_w, but *P.aurantiogriseum* was dominant after 4 months. After 6 months storage *S.roseus* was isolated from 20% of straw segments, but only at 0.85 a_w.

Table 3.12 showed that at 15^0C *P.aurantiogriseum* also predominated over the storage period at all a_w levels investigated. In addition, *E.nigrum* was isolated after 2 months and 6 months at 0.98 and 0.90 a_w respectively. This species was not present at 10^0C. After 6 months *T.harzianum* was also isolated at 0.95 and 0.98 a_w.

Table 3.10 The effect of the interaction between temperature, a_w and storage period on total fungal populations (x 10^2 CFU g^{-1} straw)

a_w	Mean Total CFU g^{-1} straw (x 10^2)								
	10^0C			15^0C			25^0C		
	2 mths	4 mths	6 mths	2 mths	4 mths	6 mths	2 mths	4 mths	6 mths
0.85	3	20	23	50	50	50	≥100	75	79
0.90	50	25	50	70	≥100	≥100	≥100	≥100	≥100
0.95	75	≥100	≥100	75	≥100	≥100	≥100	≥100	≥100
0.98	75	25	81	≥100	50	≥100	≥100	97	≥100

Table 3.11 The mean percentage isolation of species over a range of a_w levels from straw segments stored at 10^0C.

Species	Mean percentage isolation											
	10^0C, 2 months				10^0C, 4 months				10^0C, 6 months			
	0.85	0.90	0.95	0.98	0.85	0.90	0.95	0.98	0.85	0.90	0.95	0.98
Penicillium aurantiogriseum	80	100	100	100	100	73.3	100	46.6	65	100	75	75
S.roseus	20	0	0	0	0	0	0	0	20	0	0	0
T.harzianum	0	0	0	0	0	0	0	0	15	0	0	0
P.hordei	0	0	0	0	0	0	0	0	0	0	25	25

Table 3.12 The mean percentage isolation of species over a range of a_w levels from straw segments stored at 15^{0}C.

Species	Mean percentage isolation											
	15^{0}C, 2 months				15^{0}C, 4 months				15^{0}C, 6 months			
	0.85	**0.90**	**0.95**	**0.98**	**0.85**	**0.90**	**0.95**	**0.98**	**0.85**	**0.90**	**0.95**	**0.98**
Penicillium aurantiogriseum	100	100	100	71	100	100	100	100	100	71	68	83
E.nigrum	0	0	0	29	0	0	0	0	0	29	0	0
T.harzianum	0	0	0	0	0	0	0	0	0	0	18	17
P.hordei	0	0	0	0	0	0	0	0	0	0	14	0

Table 3.13 showed that *P.aurantiogriseum* was still the dominant species over the storage period except at 0.85 a_w after 2 months, when *P.aurantiogriseum* was not present and after 4 months at 0.95 a_w when *E.nigrum* was as frequently isolated. *E.amstelodami* was isolated for the first time and was dominant at 0.85 a_w after 2 months of storage. The three Tables together (3.11, 3.12, 3.13) demonstrate the effect of temperature on fungal succession of straw; as storage temperature increased the number of different species increased. This trend was observed at each a_w investigated.

The general trend observed from Table 3.14 was that for each of the temperatures as the a_w increased the percentage DML increased over the storage period.

3.4 USE OF BIOCIDES TO CONTROL FUNGAL ACTIVITY ON STRAW

3.4.1 In vitro studies on efficacy of biocides over the temperature range 10-30^{0}C on fungal activity

The biocides (Table 2.5) were incorporated into MEA media for in vitro studies on the efficacy against the fungi colonising straw over the temperature range 10-30^{0}C.

3.4.2 Control of natural mycoflora of straw using biocides

All of the biocides investigated reduced fungal isolation when compared to the control, regardless of concentration. For each of the biocides at 100 ppm total inhibition of fungal growth was achieved at each of the temperatures investigated.

Table 3.13 The mean percentage isolation of species over a range of a_w levels from straw segments stored at 25^{0}C.

Species	Mean percentage isolation											
	25^{0}C, 2 months				25^{0}C, 4 months				25^{0}C, 6 months			
	0.85	**0.90**	**0.95**	**0.98**	**0.85**	**0.90**	**0.95**	**0.98**	**0.85**	**0.90**	**0.95**	**0.98**
Penicillium aurantiogriseum	0	100	58	54	71	56	50	46	63	47	63	65
E.amstelodami	79	0	0	0	0	0	0	0	0	0	0	0
R.pusillus	21	0	0	0	29	0	0	0	0	0	0	0
E.nigrum	0	0	23	46	0	44	50	18	37	25	37	35
Cladosporium cladosporioides	0	0	19	0	0	0	0	0	0	0	0	0
A.niger	0	0	0	0	0	0	0	36	0	28	0	0

Table 3.14 The calculated dry matter loss after a range of storage periods at a range of different temperatures and water availability's.

	Mean percentage DML (g^{-1} straw)											
Temp. ^0C	**2 months**				**4 months**				**6 months**			
	0.85	**0.90**	**0.95**	**0.98**	**0.85**	**0.90**	**0.95**	**0.98**	**0.85**	**0.90**	**0.95**	**0.98**
10	15	25	28	31	24	24	26	26	20	21	24	42
15	17	22	24	46	22	38	17	40	19	46	38	45
25	16	19	32	35	21	25	28	29	18	21	25	24

Table 3.15 showed the effect of the interaction between biocide concentration and temperature. The table showed the efficacy of Busan 881 at various concentrations. Complete inhibition of fungal growth occurred at 100 ppm concentration regardless of temperature. Generally, as temperature was increased the percentage isolation of fungi increased, this occurred for all concentrations. The fungi isolated decreased with increasing biocide concentration, therefore fungal colonisation and growth was affected by concentration. The same trend was seen for the other biocides investigated. Although results not shown, Lastil 40 had a greater efficacy in inhibiting fungal isolation at all concentrations.

3.4.3 Efficacy of biocides on controlling spore germination

Each of the biocides incorporated into the media, regardless of concentration, was unable to prevent germination of spores. Each of the species had 100% spore germination on the control media, and 100% germination on the biocide-adjusted media. Concentration of biocide did not affect the amount of germination at all temperatures investigated.

3.4.4 Biocide efficacy against mycelial growth of fungi

Growth of the fungi was determined by measuring the radius of the colony and from this data the growth rates were calculated from the gradient of the lines, measured in mm day^{-1}. The growth of fungi on biocide-adjusted media were compared to the untreated controls.

Table 3.15 Mean percentage isolation of fungi from straw segments on Busan 881-adjusted media at various temperatures incubated for 7 days.

Concentration (ppm)	Mean percentage isolation			
	10^0C	15^0C	25^0C	30^0C
0.8	40	60	80	100
8	40	40	60	80
80	20	20	20	40
100	0	0	0	0
Control	80	100	100	100

Figure 3.16 shows that growth was significantly reduced when fungi were grown on media containing Busan 881 (8 ppm), However, it was less effective at controlling *T.harzianum* and *M.pusillus*, their radial growth was reduced by 50 %.

Figure 3.17 demonstrates the effect of 0.8 ppm Busan 881 on growth of *E.nigrum* when compared to growth on MEA at 25^0C. Generally growth was reduced by 50% by the biocide. The inhibition occurred throughout the 7 day growth period.

The general trend for all species is shown in Figure 3.18. As the biocide concentration was increased, the radial growth rate for each species decreased. All of the biocide concentrations were effective at controlling radial growth when compared to the control (MEA). Busan 881 was least effective at controlling the growth rate of *T.harzianum* and *M.pusillus*.

3.4.5 Control of fungal respiratory activity on straw using biocides

The effect of various concentrations of Busan 881 on respiratory activity on straw is shown in Figure 3.19. This demonstrates that over the storage period Busan 881 at 80 ppm concentration offered the most effective control of fungal activity. After 168 hours of storage, Busan 881 (80 ppm) inhibited fungal respiration from 7500 ml Kg^{-1} of straw (control) to 3100 ml Kg^{-1} of straw. The efficiency of the biocide increased over time for each of the concentrations. However, the storage period was limited to only seven days.

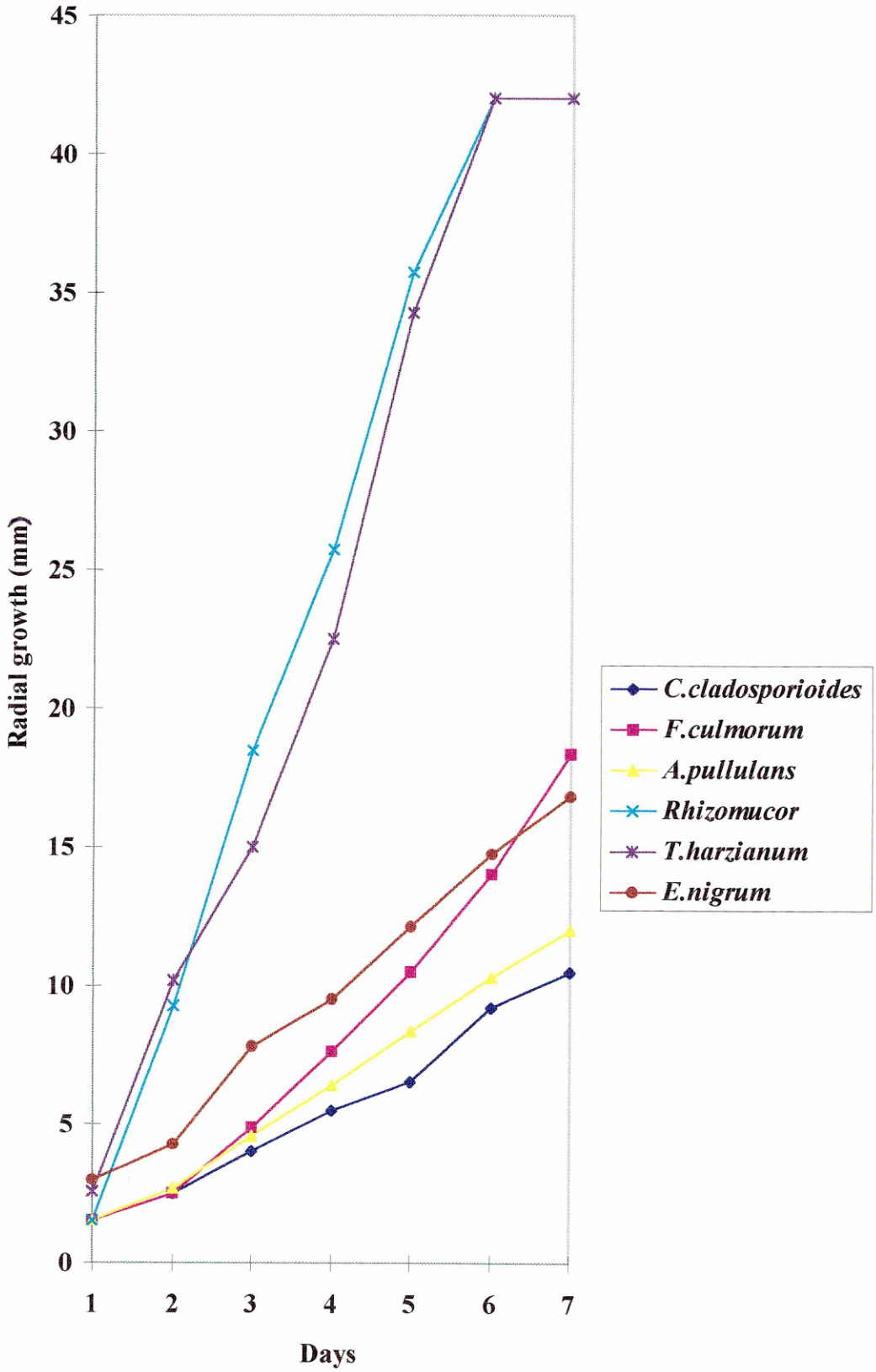


Figure 3.16 The in vitro effect of Busan 881 concentration (8 ppm) on fungal growth of six different fungal species incubated at 15^0C.

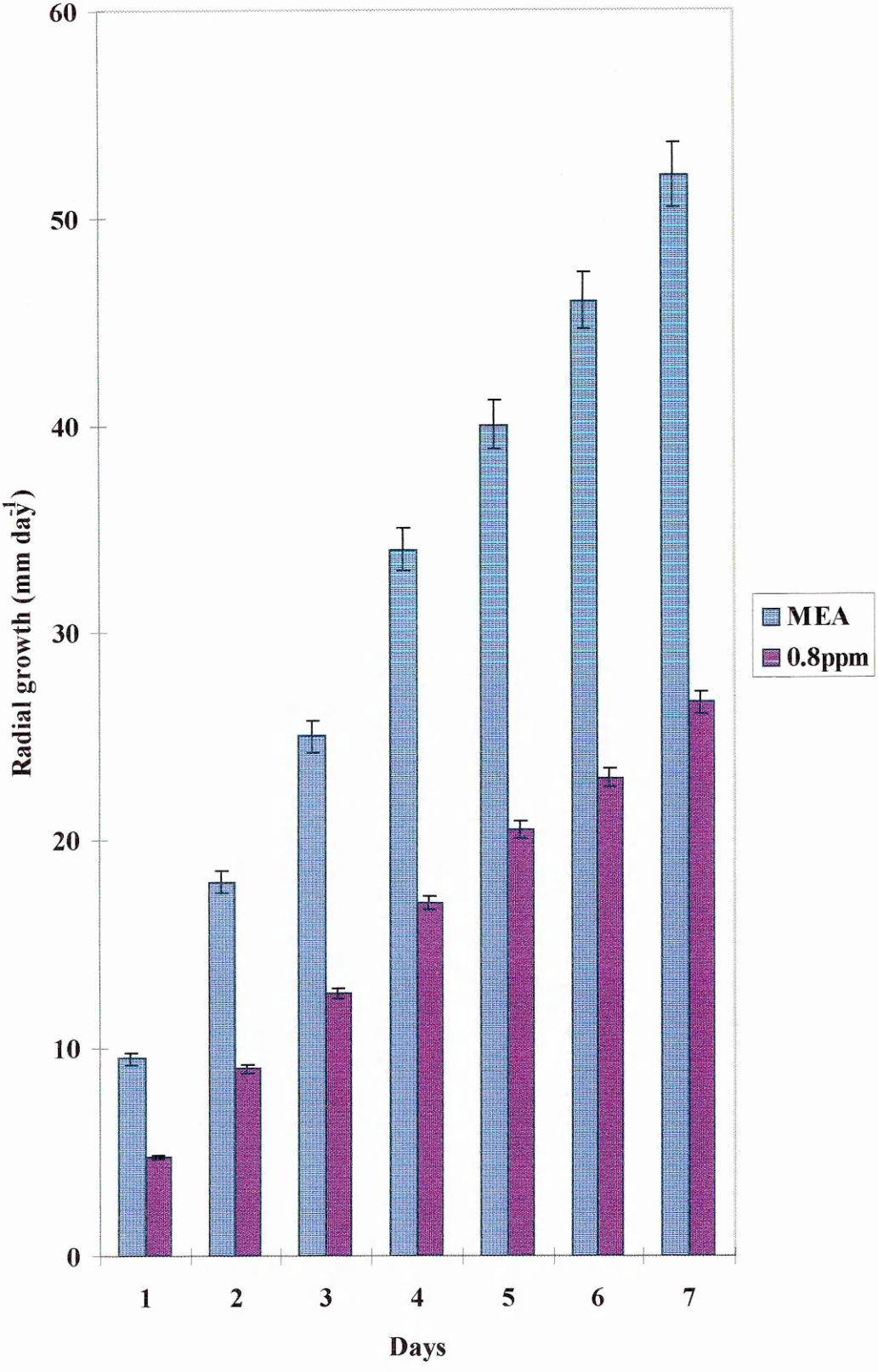


Figure 3.17 Comparison of the effect of Busan 881 (0.8 ppm) on growth of *E. nigrum* at 25^{0}C when compared to untreated control.

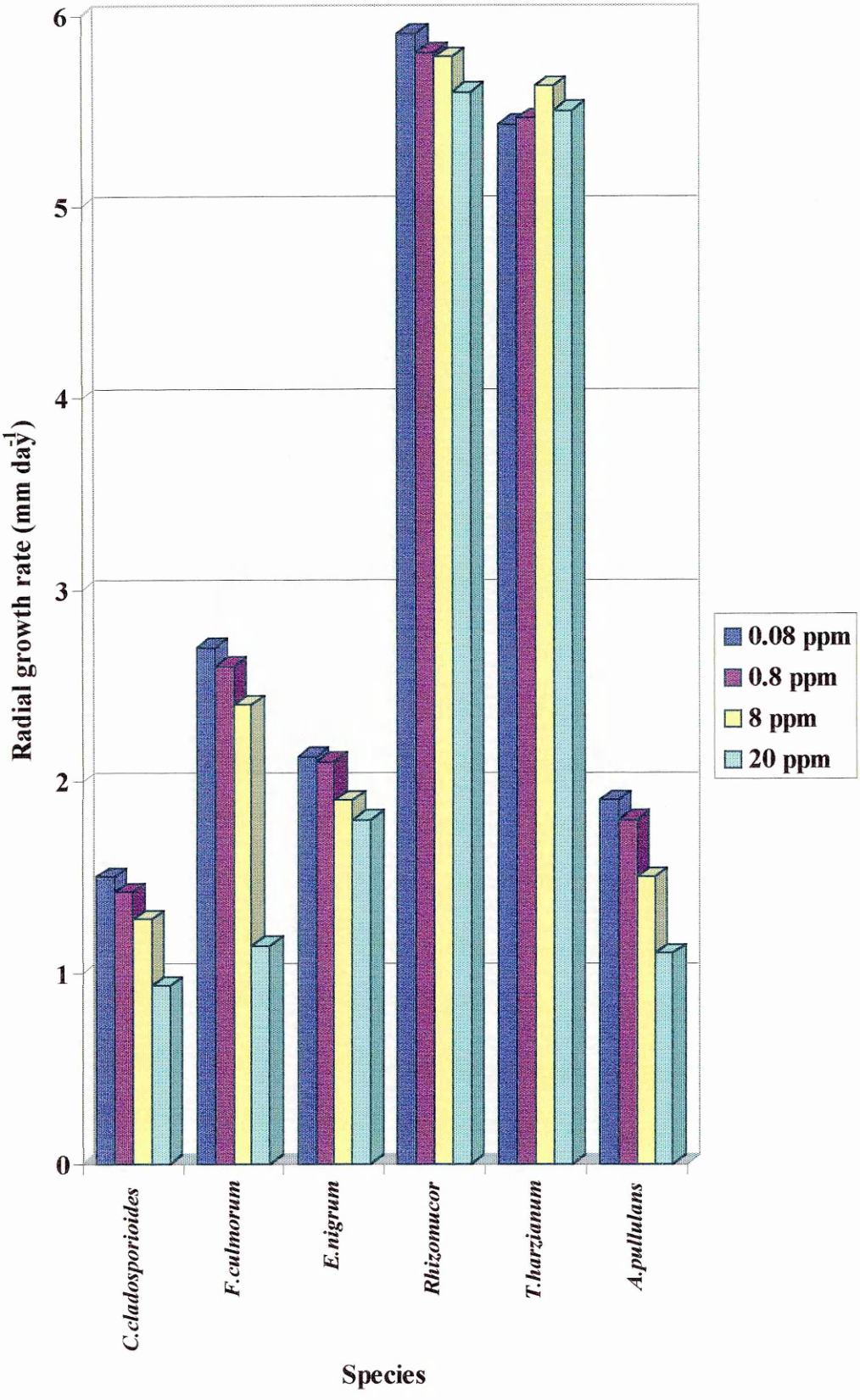


Figure 3.18 Comparison of the effect of various concentrations of biocides on growth rates of fungi (mm day^{-1}) at 15^{0}C.

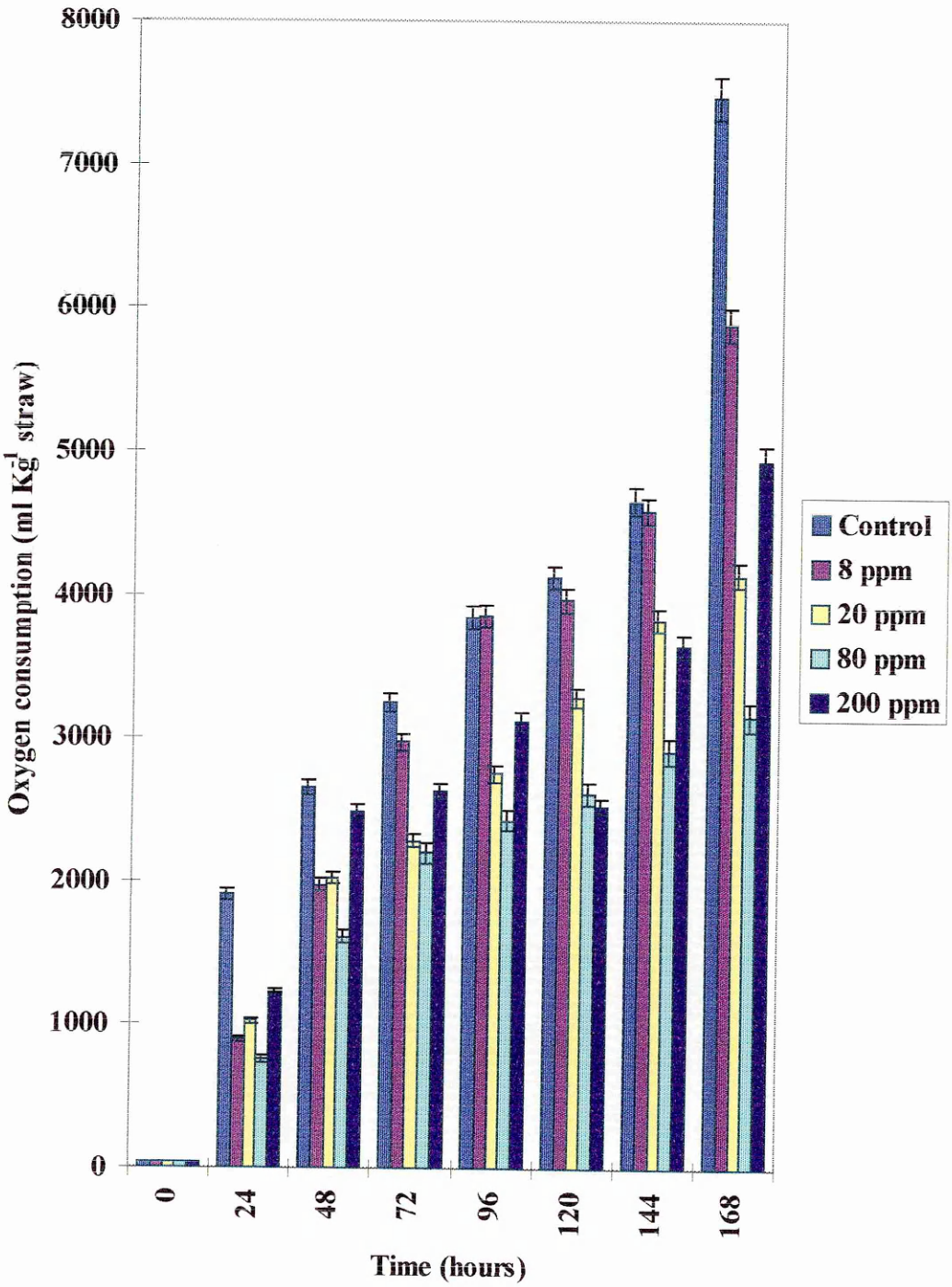


Figure 3.19 The effect of Busan 881 at various concentrations on fungal respiration on wheat straw adjusted to 0.95 a_w after 7 days of storage at 25^0C (cv.Ribband).

Figure 3.20 shows that at 0.98 a_w fungal respiration was dramatically increased on the untreated control straw, over 50% compared to fungal respiration at 0.95 a_w (Figure 3.19), indicating that respiratory activity is influenced by a_w. The efficacy of Busan 881 was slightly diminished with the increase in a_w, it was more effective at 0.95 a_w than at 0.98 a_w, therefore the biocide efficacy was a_w dependant.

The efficacy of a range of different biocides at various concentrations on controlling fungal respiration on straw is shown in Figure 3.21. All of the biocides controlled fungal respiration. The most effective biocide was Lastil 40 at a concentration of 100 ppm, which decreased fungal respiration by 66% when compared to the control straw. Increasing the concentration of Busan 881 did not increase the efficacy in controlling fungal respiration, which was unexpected.

Table 3.16 showed the dry matter losses during storage. All of the biocides reduced the DML when compared to the control at both a_ws. Straw treated with Lastil 40 was the most effective treatment for reducing DML.

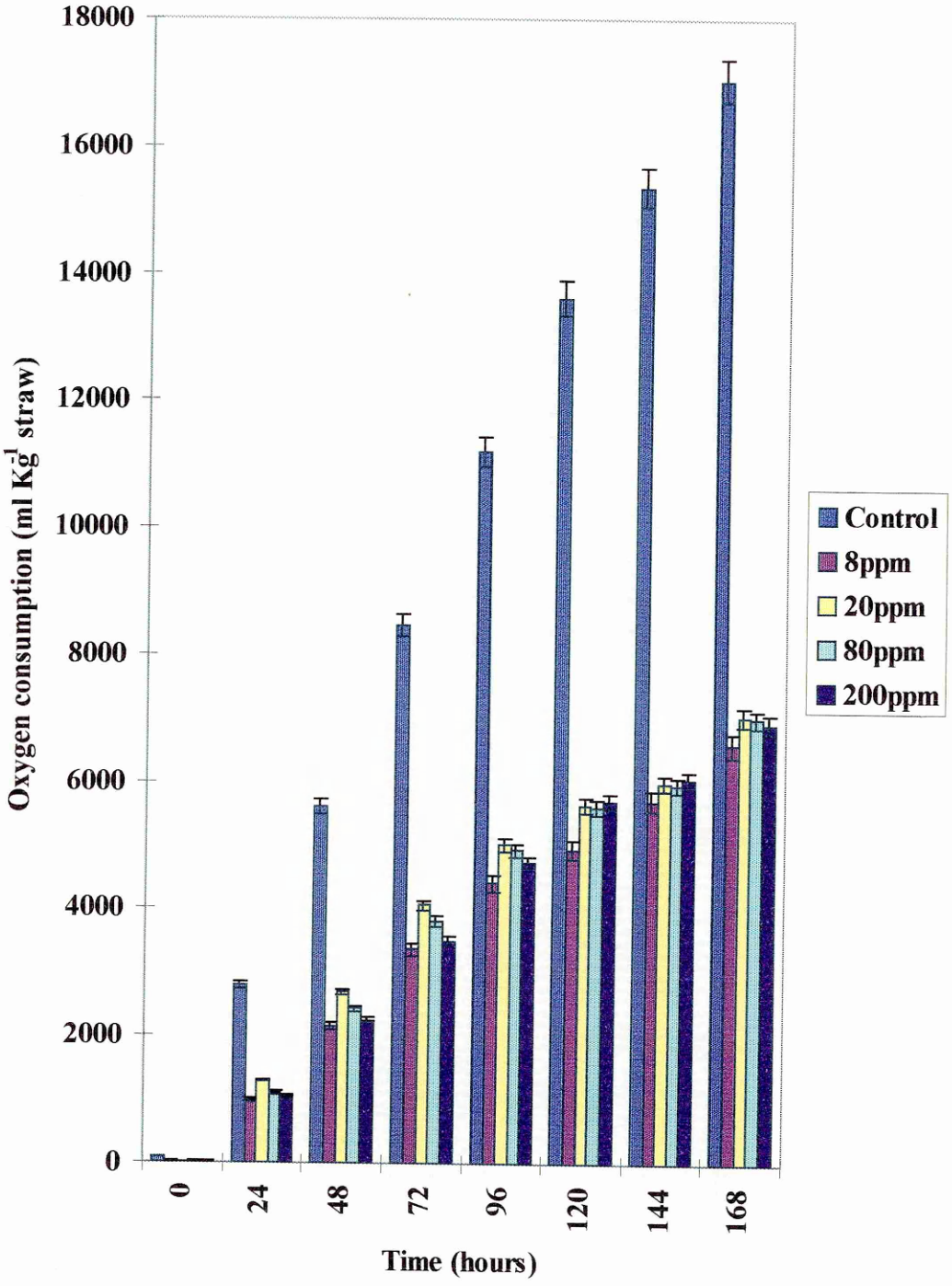


Figure 3.20 The effect of Busan 881 at various concentrations on fungal respiration on wheat straw adjusted to 0.98 a_w after 7 days of storage at 25^0C (cv.Ribband).

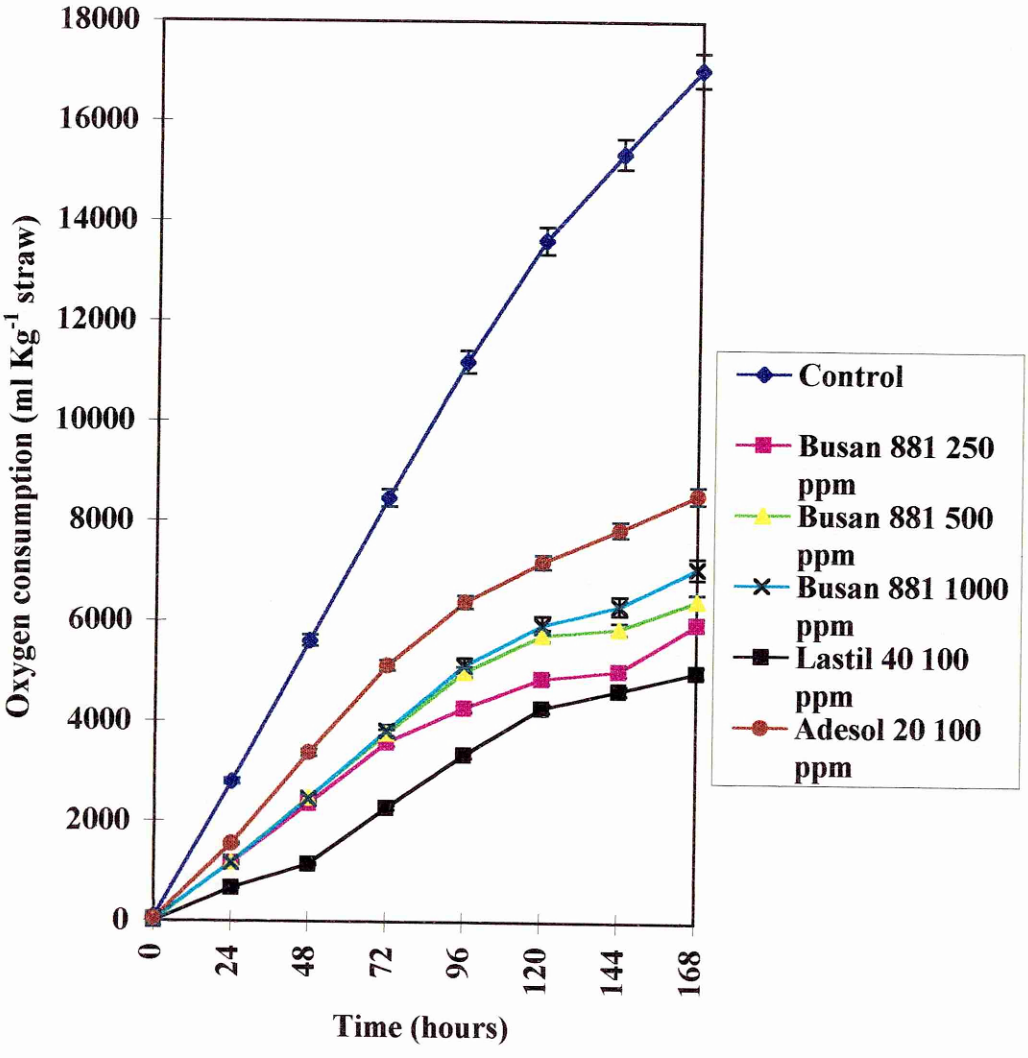


Figure 3.21 The effect of various biocides on fungal respiration on wheat straw adjusted to 0.98 a_w after 7 days of storage at 25^0C (cv.Ribband).

Table 3.16 The effect of a_w and biocide treatment on calculated DML in wheat straw after 14 days incubation.

Treatment	DML (%)	
	a_w	
	0.95	**0.98**
Control	0.74	0.81
Busan 881, 250 ppm	0.32	0.39
Busan 881, 500 ppm	0.37	0.43
Busan 881, 1000 ppm	0.41	0.47
Lastil 40, 100 ppm	0.26	0.33
Adesol 20, 100 ppm	0.50	0.57

3.5 FIELD EXPERIMENTS

3.5.1 Experiment to determine efficacy of various biocides (October 1995-March 1996) (NS2)

Three biocides which were found to be effective in laboratory studies were tested on whole bales. The biocides were Adesol 20 (100 ppm, Buckman Labs), Busan 881 (250 ppm, Buckman Labs) and Lastil 40 (100 ppm, Coalite Chemicals). Water was sprayed onto the surface of the control bales. Samples were taken at monthly intervals and the fungal populations evaluated from six areas within the bale as described previously in Section 2.8.1.

Table 3.17 showed that the mean fungal total population varies depending on the area that was sampled. This demonstrates the importance of sampling in a similar location at each sampling time. Generally, all the biocides tested reduced the mean total fungal populations isolated from treated straw when compared to untreated controls. However, the efficacy of the biocides varied. Straw treated with Lastil 40 had lower total fungal populations when compared to the control regardless of sampling location. Lastil 40 generally had the greatest efficacy when compared to the other biocide treatments. An exception to this observation was samples from the front left which were treated with Adesol 20. The straw had 46 x 10^2 CFU g^{-1} straw, less than straw treated with Lastil 40. However, 25 % of the colonies isolated from straw treated with Adesol 20 were *A.fumigatus* which is a

Table 3.17 The effect of sampling location and efficacy of biocides on mean total fungal population of straw bales after 180 days of storage.

Treatment	Mean total fungal population (x 10^2 CFU g^{-1} straw)					
	Front right	Back right	Front left	Back left	Middle	Side of bale
Control	≥100	≥100	≥100	76	77	76
Adesol 20	75	64	46	≥100	≥100	32
Busan 881	≥100	83	78	≥100	29	87
Lastil 40	67	79	52	75	75	52

respiratory allergen and poses a health risk to people who inhale these spores compared to only 2 x 10^2 CFU g^{-1} straw from that treated with Lastil 40. Consequently, although Adesol 20-treated straw in this instance resulted in lower fungal populations than Lastil 40-treated straw, the predominant species could cause serious health problems to people coming into contact with the straw.

Figure 3.22 shows that each of the biocides controlled microbial growth compared to the control. The dominant species isolated from the control samples were *A.fumigatus* (50 x 10^2 CFU g^{-1} straw) and *F.culmorum* (50 x 10^2 CFU g^{-1} straw). All of the biocide-treated straw had significantly less (P <0.05) *A.fumigatus* populations with Adesol 20 and Lastil 40 treatments having means of 10^2 CFU g^{-1} straw and Busan 881-treated straw having no *A.fumigatus* present. The figure showed that Adesol 20 had the lowest fungal populations. However, 42 % of the total were *T.harzianum* which is a cellulose degrading fungus which may affect the straw structure. Straw treated with Lastil 40 did not have any of this species present. Therefore, although it had higher fungal populations than Adesol 20-treated straw it appeared to have more potential as a treatment for conserving straw quality.

The mean total fungal populations in core samples were lowest from that treated with Lastil 40, when compared to the control and other biocide treatments (Figure 3.23). In the Lastil 40 treated straw *E.amstelodami* was the dominant spoilage fungus present.

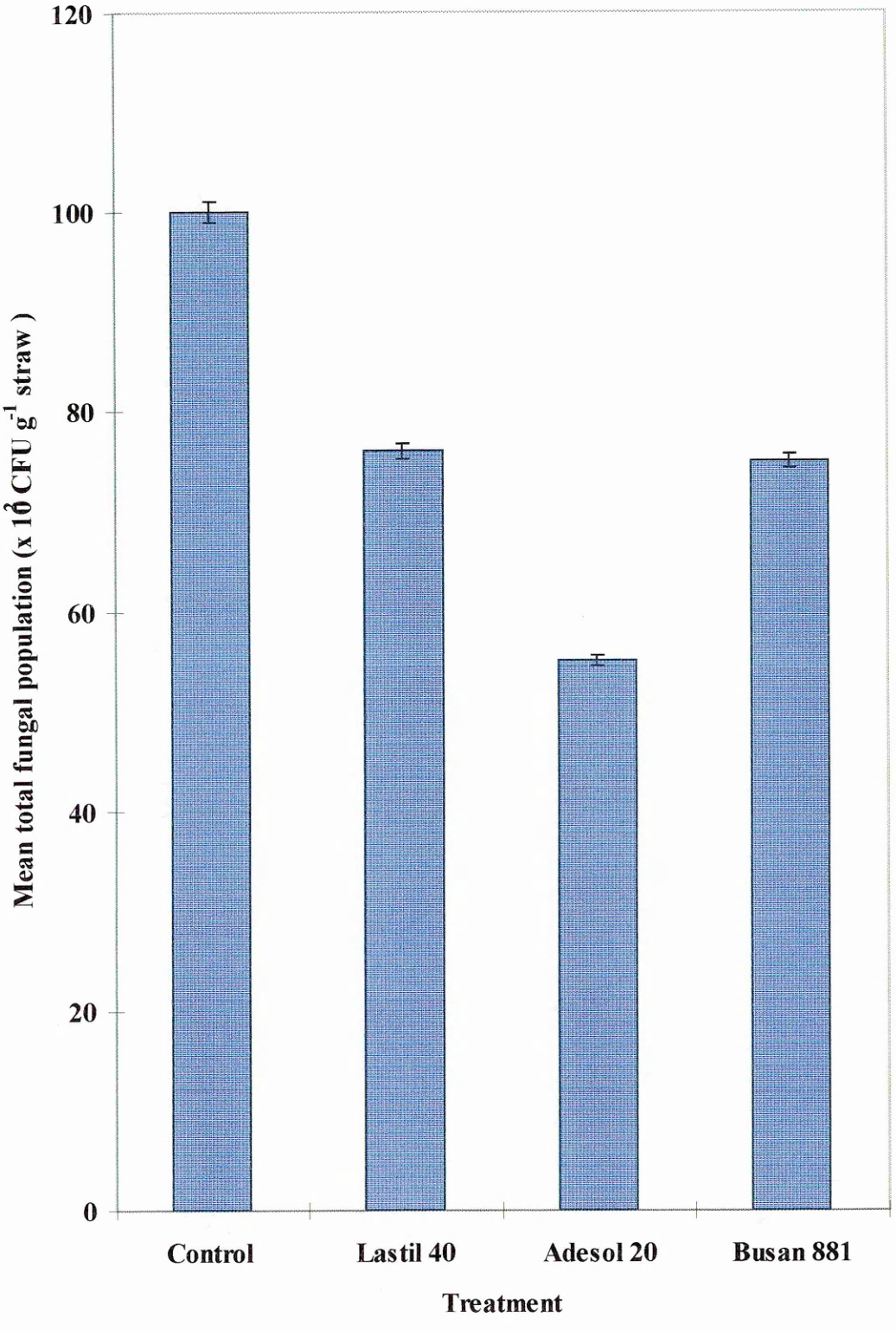


Figure 3.22 The effect of biocides on fungal populations isolated from the edge samples from the top bales of straw after 180 days storage.

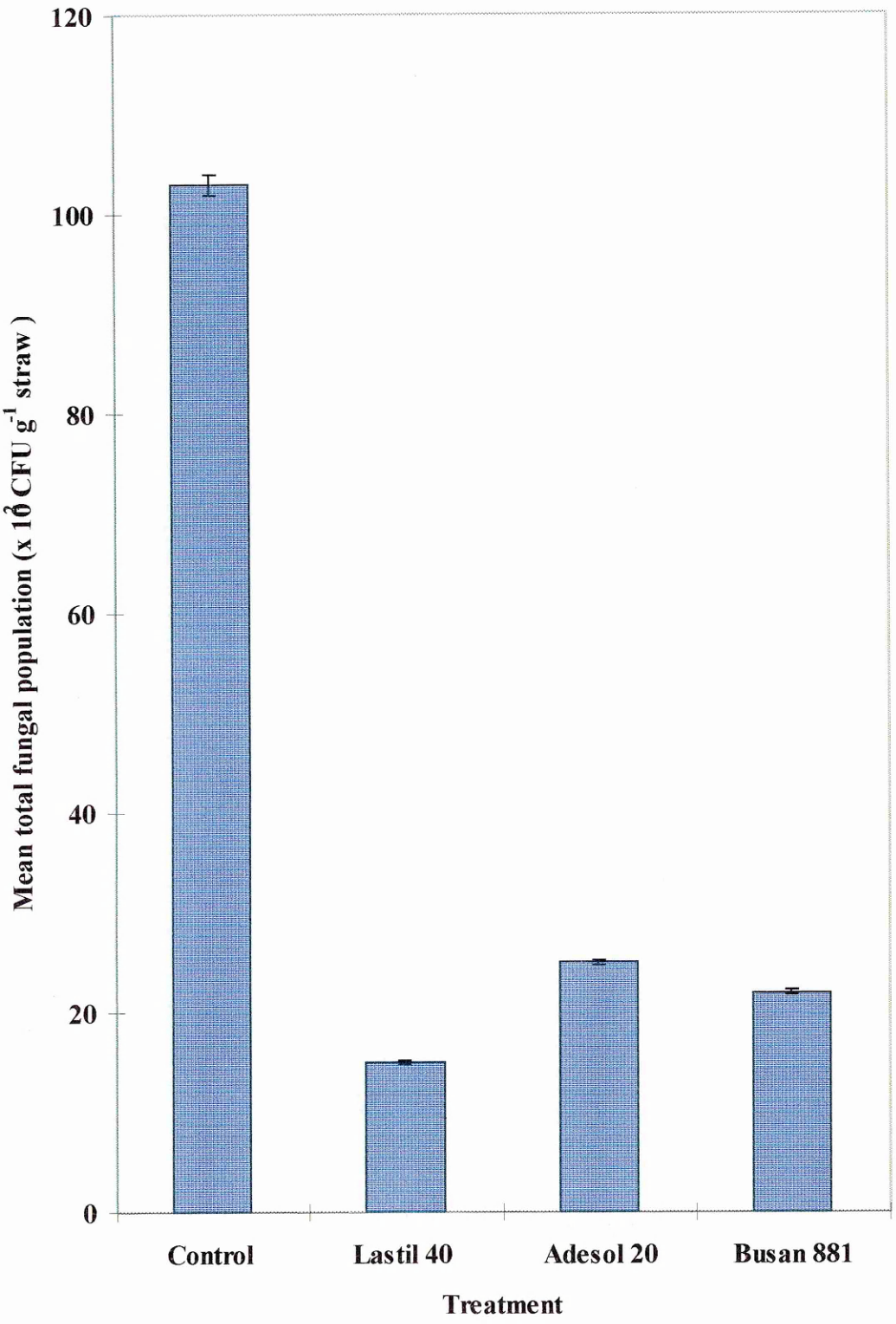


Figure 3.23 The effect of biocides on fungal populations isolated from the core samples from the top bales of straw after 180 days storage.

Table 3.18 showed that both control bales were not significantly different with regards to NCP content. However, bale1 had a slightly higher lignin content. Storage for six months did produce changes regardless of treatment. Comparison of the core (A) of the control bale 1 and the corresponding edge of the bale (B) showed that there was a change in lignin and NCP content from 20.6 and 33.0 % to 24.7 and 16.9 %, respectively. For bale 2, storage was accompanied by an apparent increase and decrease in lignin and NCP contents respectively, although neither change was statistically significant.

Treatment with Adesol 20 resulted in no significant changes in the lignin content of the inner or outer bale samples. However, both experienced a reduction in NCP content to 18 and 17.2 %, respectively. Treatment of bale 1 with Adesol 20 produced a reduction in the NCP content in the outer area of the bale (F) to 16.5 % with no change in the NCP content of the inner bale (E) or in the lignin content of either the inner or outer bale. Treatment with Lastil 40 only produced small but significant reductions in NCP to 23.5 % and 26.6 %. The lignin contents did not change significantly. After treatment with Busan 881, the lignin and NCP contents of bale1 samples decreased to 15.2 and 26.4 % respectively. Treatment of both bales with Busan 881 for six months inhibited NCP degradation and resulted in samples (N and P) with compositions approaching that of the control samples. Based on the chemical data these experiment indicated that Lastil 40 and Busan 881 were superior to Adesol 20 in inhibiting the degradation of cereal straw when stored for six months. Although changes in the apparent lignin content occurred, these were probably due to losses of carbohydrate components. Some modification

Table 3.18 The changes in lignin and non-cellulosic polysaccharide content of straw treated with different biocides after six months storage.

Sample	Description	Position	Lignin (%)	NCP (%)
A	Control Bale 1	Core	20.6 (2.6)	33.0 (3.7)
B		Edge	24.7 (3.1)	16.9 (2.3)
C	Control Bale 2	Core	18.8 (0.6)	30.0 (1.5)
D		Edge	21.0 (2.9)	21.7 (9.4)
E	Adesol 20 Bale 1	Edge	20.3 (2.6)	28.4 (4.1)
F		Core	22.7 (3.2)	16.5 (3.2)
G	Adesol 20 Bale 2	Edge	17.4 (2.2)	18.0 (7.2)
H		Core	20.8 (3.1)	17.2 (6.6)
I	Lastil 40 Bale 1	Edge	19.4 (1.6)	23.5 (5.6)
J		Core	21.7 (2.0)	26.6 (5.6)
K	Lastil 40 Bale	Edge	17.3 (2.6)	27.6 (11.2)

	2			
L		Core	20.7 (2.9)	24.6 (1.0)
M	Busan 881 Bale 1	Edge	15.2 (1.8)	26.4 (1.0)
N		Core	19.8 (1.5)	30.1 (1.8)
O	Busan 881 Bale 2	Edge	16.6 (3.6)	31.3 (7.4)
P		Core	19.6 (3.7)	25.0 (7.0)

of the lignin was observed from DRIFT spectroscopy (results not shown) but the modifications were less with Lastil 40 and Busan 881.

3.4.2 Efficacy of biocide application on re-baling of straw at various moisture contents (March 1996-August 1996) (NS3)

The field experiments in NS2 showed that the biocide Lastil 40 was one of the best treatments for the control of deterioration of straw by fungal activity. From these results it was decided to examine this biocide in a more extensive study under a range of bale moisture contents (12, 16, 20 and 24 % wet weight basis) in an experiment described in Section 2.8.2.

Table 3.19 showed that the efficacy of Lastil 40 decreased with time, regardless of moisture content. Generally the bales which were not treated with biocide had a greater number of total populations of fungi. The exception to this observation was after 150 days when the straw was adjusted to 24 % moisture content, in which both samples had 10^4 CFU g^{-1} straw. Although in this instance the biocide did not reduce the total population, the fungi isolated were different from the biocide-treated than the non-biocide-treated straw. Half of the colonies isolated from the non-biocide straw (50 x 10^2 CFU g^{-1} straw) were *A.fumigatus*, which can irritate the linings of the lungs and cause a disorder called Aspergillosis. However, the biocide-treated straw at this moisture content did not have any *A.fumigatus* present in the total fungal population. In addition, after 50 days of storage the dominant species isolated from the non-biocide-treated straw was *A.fumigatus*.

Table 3.19 The mean total fungal population of straw samples (x 10^2 CFU g^{-1} straw) from the edge of top bales under a range of moisture contents after 50, 100 and 150 days storage.

Storage period (days)	Treatment (mean total population x 10^2 CFU g^{-1} straw)							
	No biocide				Biocide-treated			
	Moisture content (wet weight basis)				Moisture content (wet weight basis)			
	12	16	20	24	12	16	20	24
50	75	84	75	≥100	50	48	54	78
100	65	80	85	90	50	70	70	80
150	77	≥100	53	≥100	75	83	50	≥100

Figure 3.24 showed that generally, the biocide (Lastil 40) reduced the mean total fungal populations found on the edge of the top straw bales after 150 days of storage compared to the non-biocide-treated bales. The biocide was effective at reducing the mean total fungal populations when the straw was adjusted to 12-20 % moisture content. At 24 % moisture content the mean total fungal populations on biocide-treated and untreated straw bales were the same (10^4 CFU g^{-1} straw). However, the species isolated were different from the two treatments. *A.fumigatus* was isolated from 5 % of untreated straw pieces, whereas the biocide-treated straw did not have any.

Generally, the biocide-treated straw reduced the mean total fungal populations compared to the untreated samples as shown in Figure 3.25. The biocide controlled fungal growth during the whole of the storage period. At 24 % moisture content after 150 days storage the mean total fungal populations on untreated straw was less than the biocide-treated bales. However, the species isolated were different from the two treatments with 4 % of untreated straw pieces having *A.fumigatus* whereas none was isolated from the biocide-treated straw. The same trend was seen for the edge samples after the same storage period.

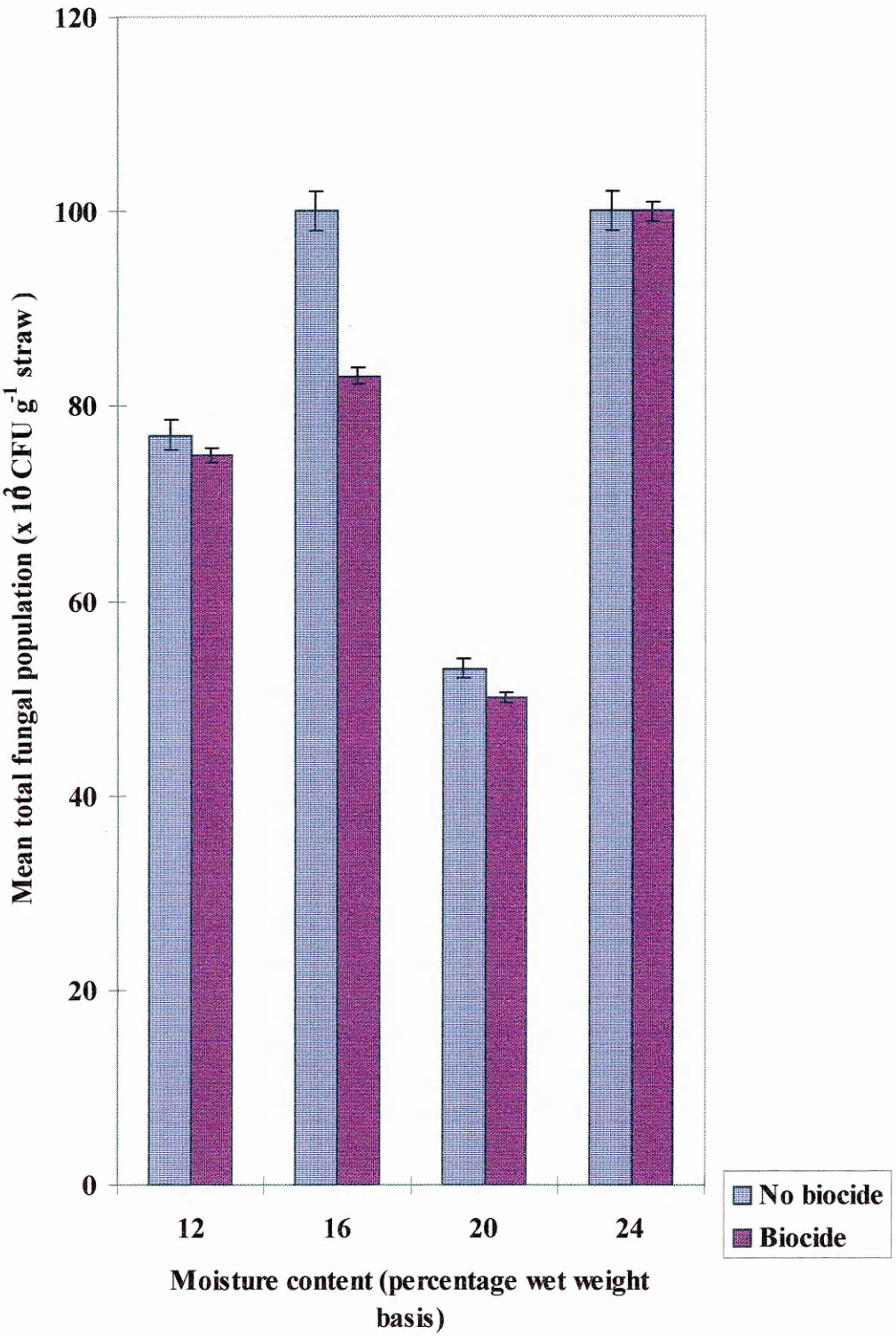


Figure 3.24 The effect of Lastil 40 (100 ppm) on fungal populations isolated from the edge samples from the top bales of straw after 150 days storage under a range of moisture contents (cv.Ribband).

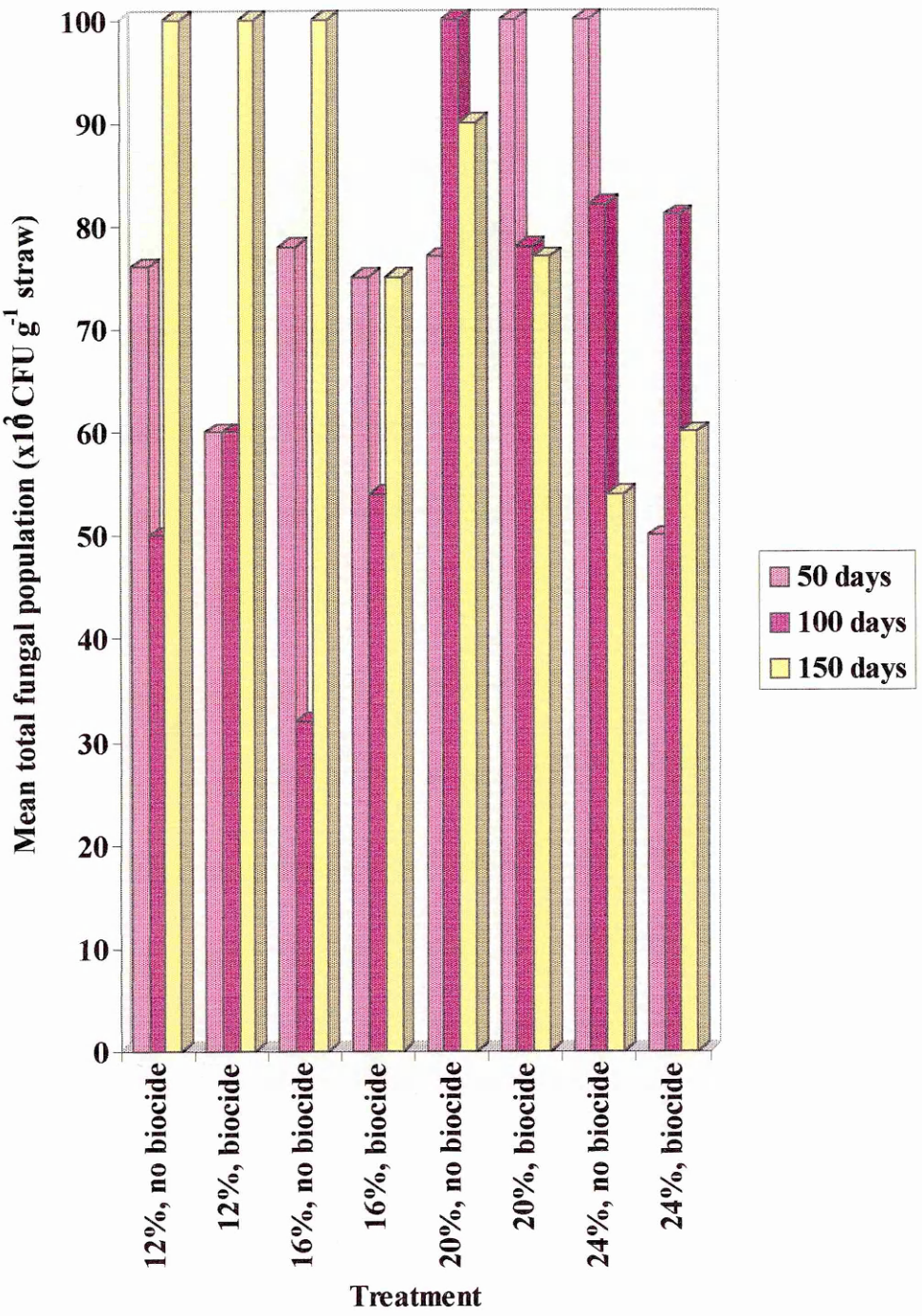


Figure 3.25 The effect of Lastil 40 (100 ppm) on fungal populations isolated from the core samples from top bales of straw during storage under a range of moisture contents (cv.Ribband).

To interpret the data and highlight the more important results the criterion of an NCP content of less than 250 g Kg^{-1} has been applied to experiments NS3 and NS4. This arbitrary figure is an NCP content approximately 30 % lower than that found in the original cereal straw sample. In the same way, to highlight the more important lignin results, the criterion of a lignin content of more than 200 g Kg^{-1} has been applied to NS3 and NS4. This arbitrary content is a level approximately 30 % higher than that found in the original cereal straw sample.

Figure 3.26 showed there were no broad trends for the NCP contents in either the untreated or biocide-treated bales. The only trend observed was that after 150 days storage the NCP content decreased compared to 50 and 100 days storage regardless of treatment. This would indicate that degradation was occurring

There were no clear trends for the NCP contents in either the untreated or biocide-treated bales which are shown in Figure 3.27. The only trend observed was that after 150 days storage the NCP content decreased when compared to 50 and 100 days storage, regardless of treatment. This would indicate that degradation was occurring. Generally, after 150 days storage the NCP content was lower in the edge samples when compared to the core samples which would indicate that more degradation was occurring in the edge samples than the core ones.

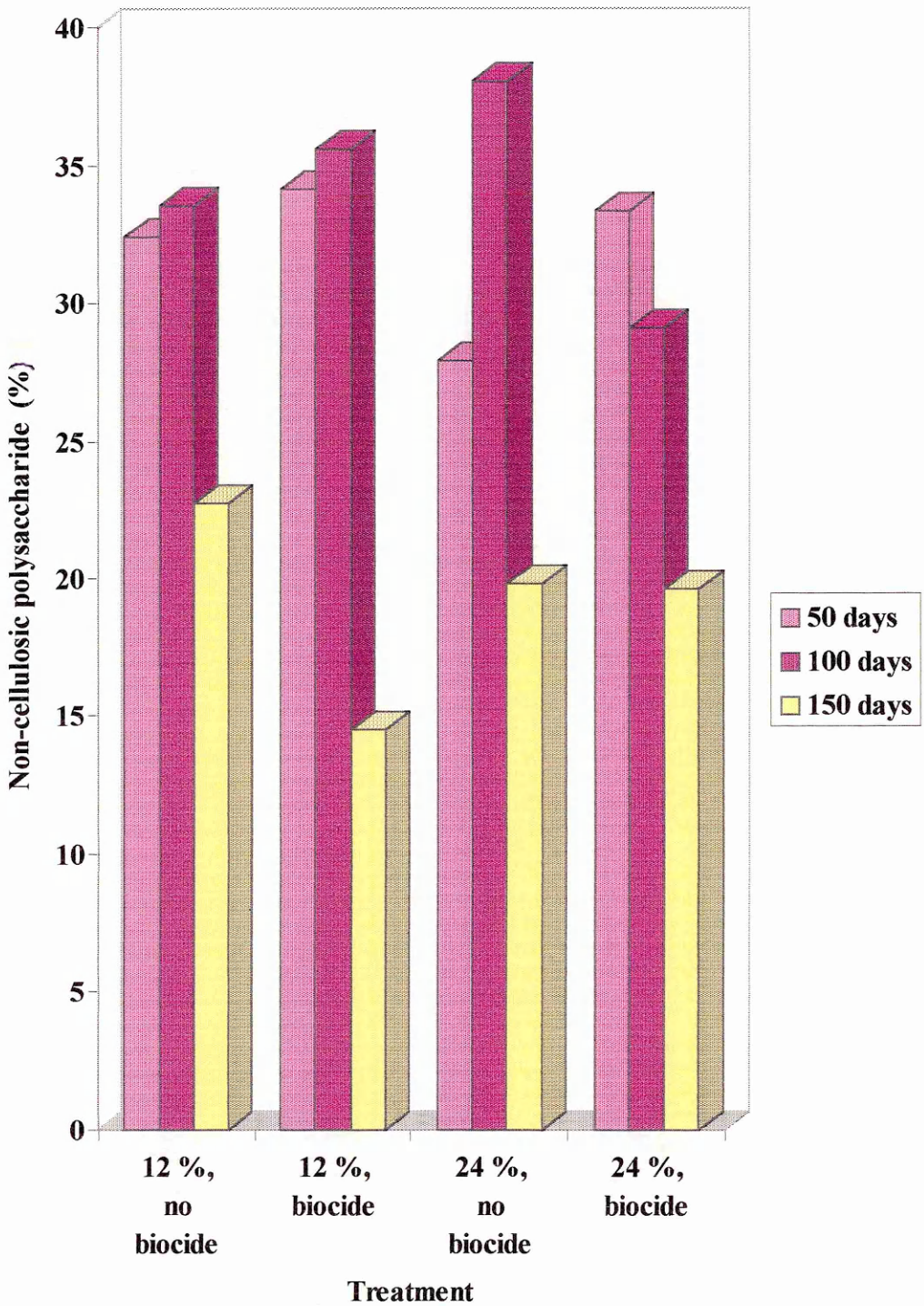


Figure 3.26 The effect of moisture content and biocide application (Lastil 40, 100 ppm) on non- cellulosic polysaccharide content of edge samples of straw from top bales during storage (cv.Ribband).

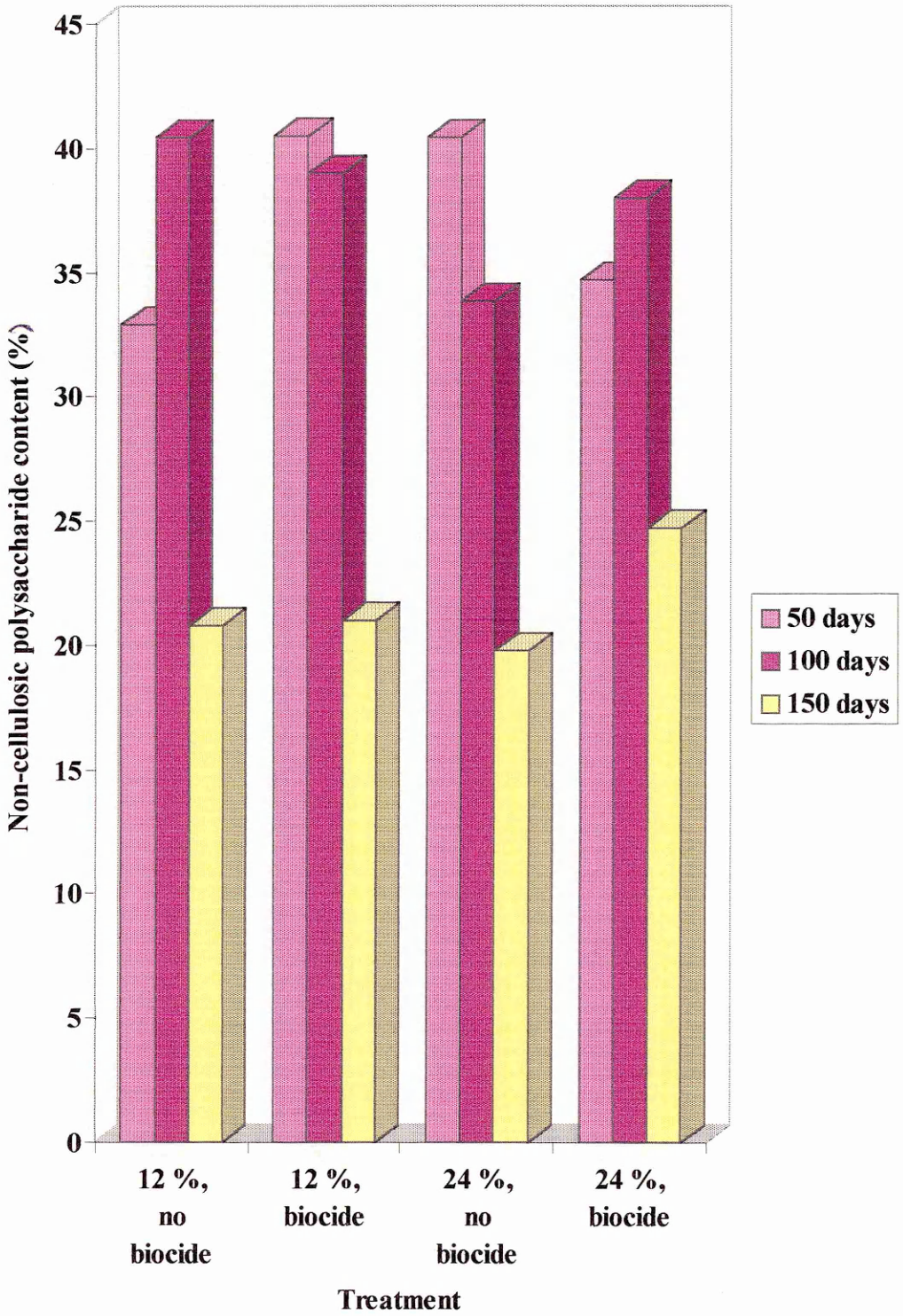


Figure 3.27 The effect of moisture content and biocide application (Lastil 40, 100 ppm) on non- cellulosic polysaccharide content of core samples of straw from top bales during storage (cv.Ribband).

Figure 3.28 showed generally that untreated straw samples had increased lignin content in the edge samples over the 150 day storage period. The general trend was also seen for the biocide-treated straw bales, particularly during storage from 100 to 150 days. Although the lignin content appears to be increasing, it is actually due to other constituents being lost to degradation and as a result the lignin is in a higher proportion of the residual straw sample.

These changes in core samples are shown in Figure 3.29. The lignin content of the core samples were always less than the lignin content of the edge samples regardless of treatment, after 150 days storage. This would indicate that greater degradation of other constituents are occurring along the edge of the straw bales than the core.

The results from the edge and core samples from middle and bottom bales were not as consistent as the trends seen from the top bales. Generally, there was a progression of increasing lignin content from bottom bale to top bale.

Analysis of the samples by DRIFT spectra showed that structural, as well as, compositional changes have taken place. An example of these spectra is shown in Figure 3.30. The fingerprint region over the range 1200-900 cm^{-1}, which is dominated by the absorbances due to cellulose and NCPs, exhibits different line shapes and maxima. A reduction in intensity centred at 1720 cm^{-1}, the region of ester carbonyl absorbance, particularly in the spectra of the edge samples. This absorbance was most intense in the spectrum of the samples with moisture contents of 12 % and at a given moisture content, greatest in the core sample.

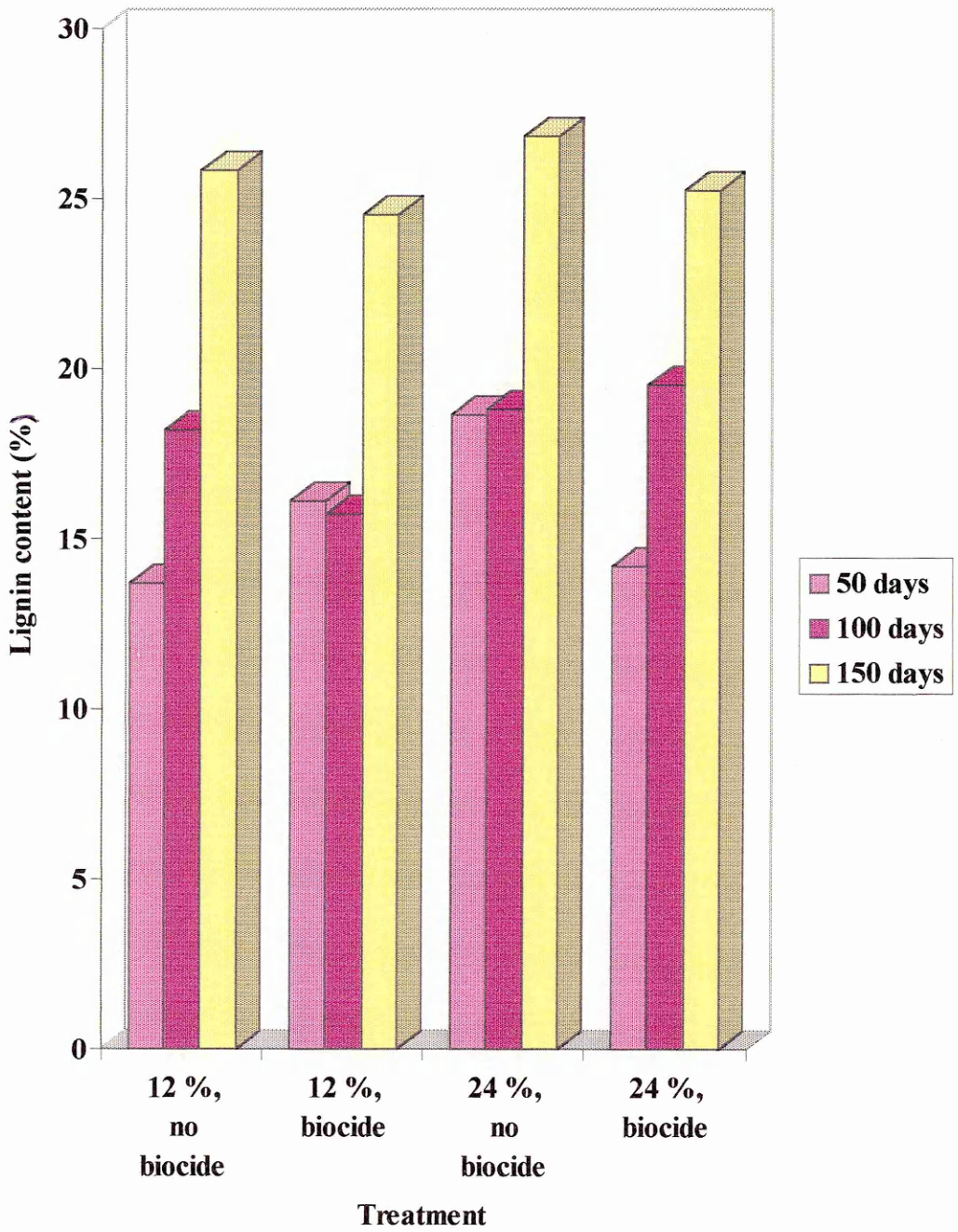


Figure 3.28 The effect of moisture content and biocide application (Lastil 40, 100 ppm) on lignin content of edge samples of straw from top bales during storage (cv.Ribband).

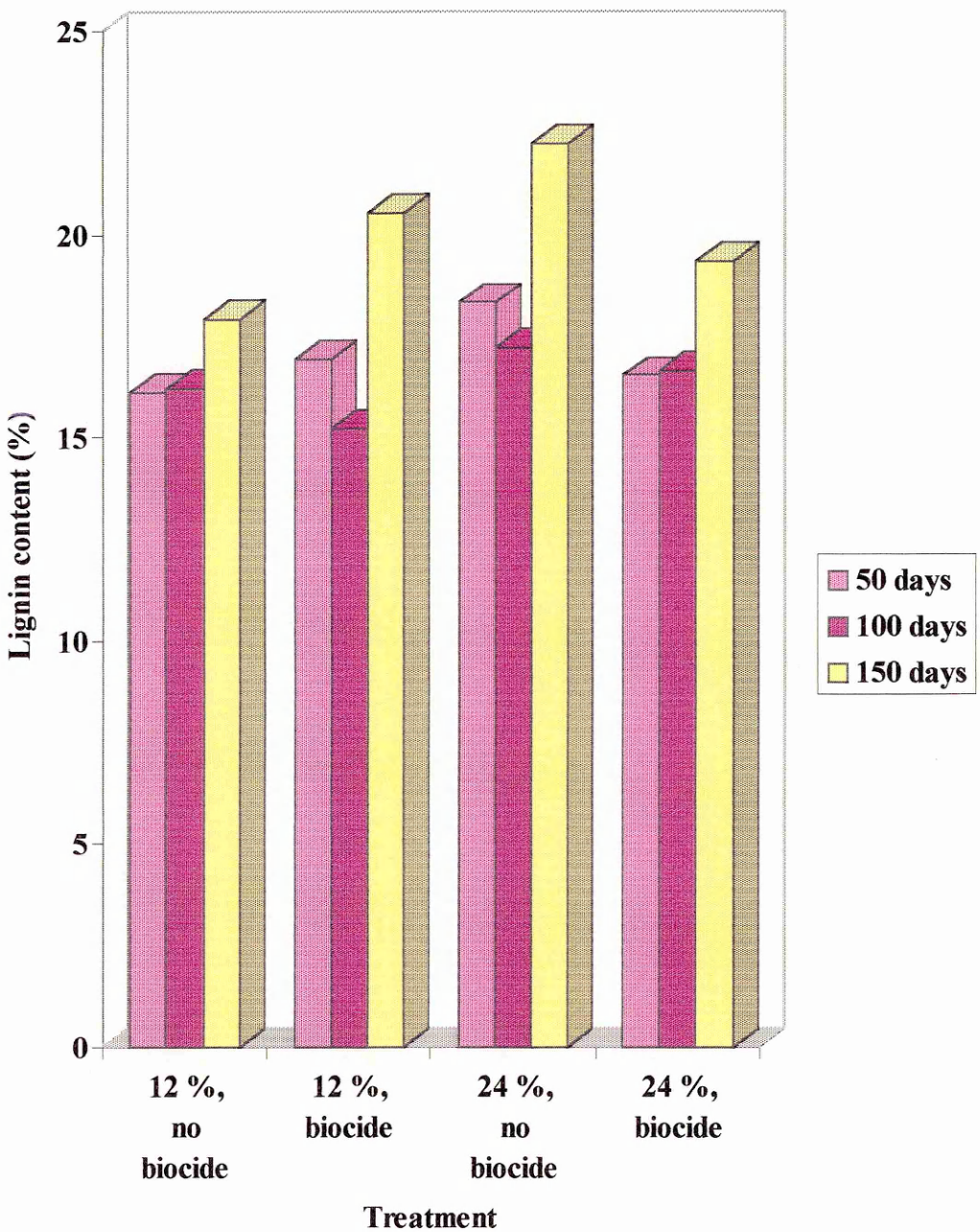
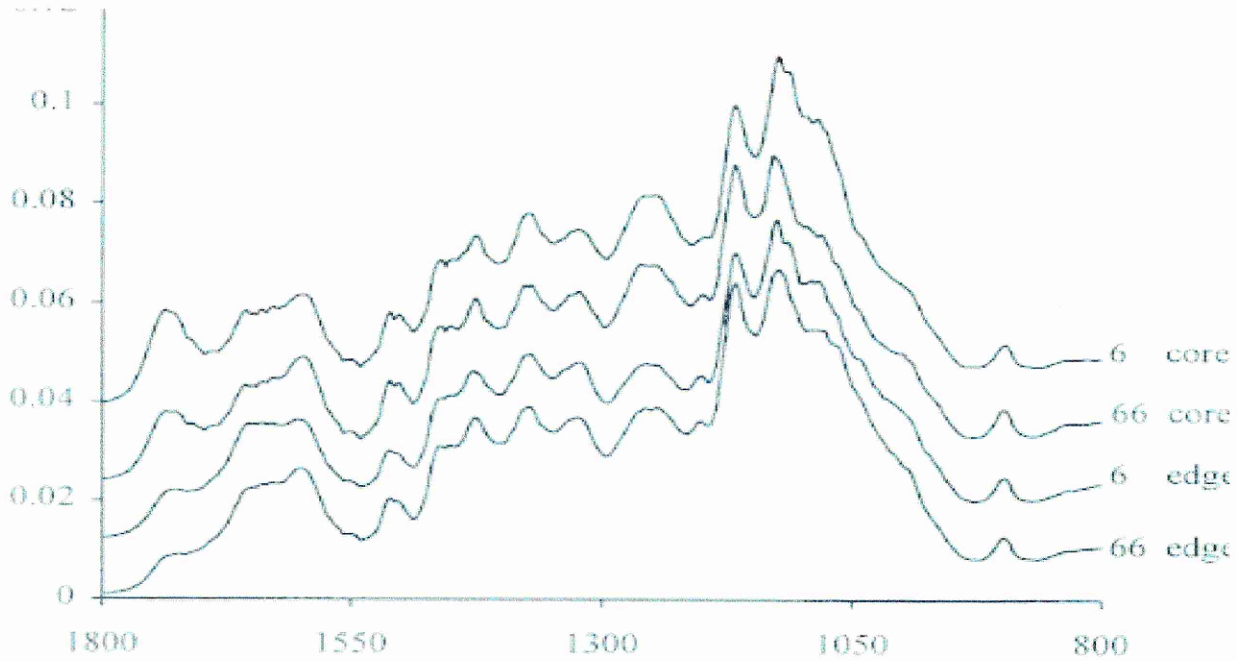


Figure 3.29 The effect of moisture content and biocide application (Lastil 40, 100 ppm) on lignin content of core samples of straw from top bales during storage (cv.Ribband).



Absorbance (y axis) **Wavenumber cm^{-1} (x axis)**

Figure 3.30 DRIFT spectra of core and edge samples from bales 6 (12 % moisture content, untreated) and 66 (24 % moisture content, untreated).

Thermocouples were inserted in all bales located at the inner two tiers of the stack, i.e. those which were extracted last, after 150 days storage. Some of the results obtained from these sensors are shown in Figures 3.31 and 3.32. This showed that there were no apparent effect that could be attributed to the biocide treatment. Figure 3.31 showed temperature peaks at intervals in the top bales, which correlates well with periods of high rainfall. The temperatures of bales below the top layer were very similar to ambient conditions which rose from 0^0C to 10^0C during the experiment.

The temperature profiles shown in Figure 3.32 show that within 10 days of the start of the experiment both biocide-treated and untreated straw increased in temperature to maximum values above 50^0C. This significantly decreased within the next 10 days. On several occasions the daily rainfall was between 7 and 12 mm (Figure 3.33) and within three days the temperatures in the upper sections of the top layer bales had risen to between 40 and 55^0C, decreasing to ambient temperature during the next 10 days. The temperature peaks in the top layer bales correlates well with periods of high rainfall. The temperatures of bales below the top layer were very similar to ambient conditions which rose from 0^0C to 10^0C during the experiment, except for several occasions when water was observed to have penetrated to both the centre and lower bale; this was noticed for untreated control bales after 50 days storage. Increases in rainfall correlated well with the temperature increases in the bales.

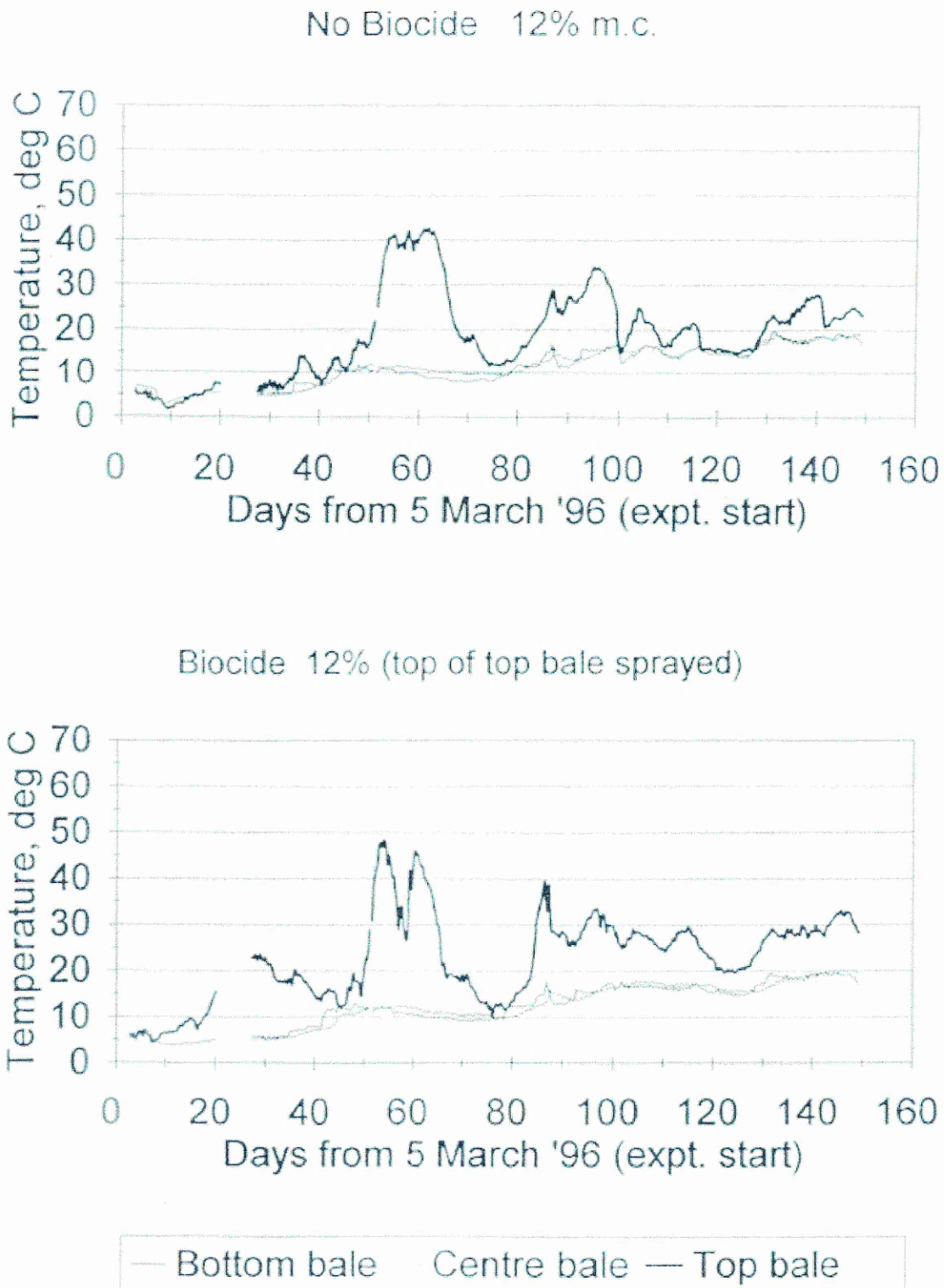


Figure 3.31 Temperature profiles of untreated and biocide-treated straw bales adjusted to 12 % moisture content during storage.

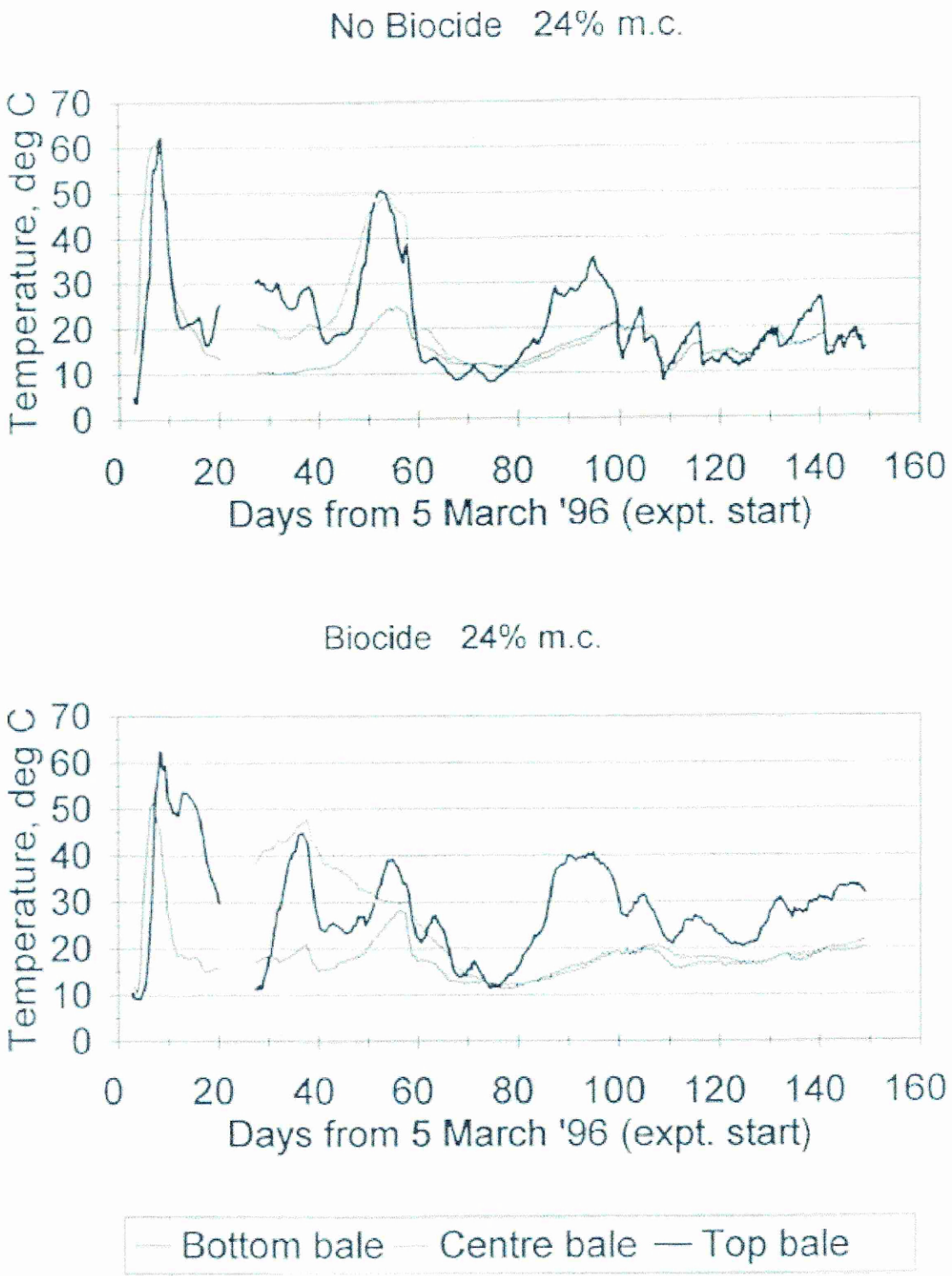


Figure 3.32 Temperature profiles of untreated and biocide-treated straw bales adjusted to 24 % moisture content during storage.

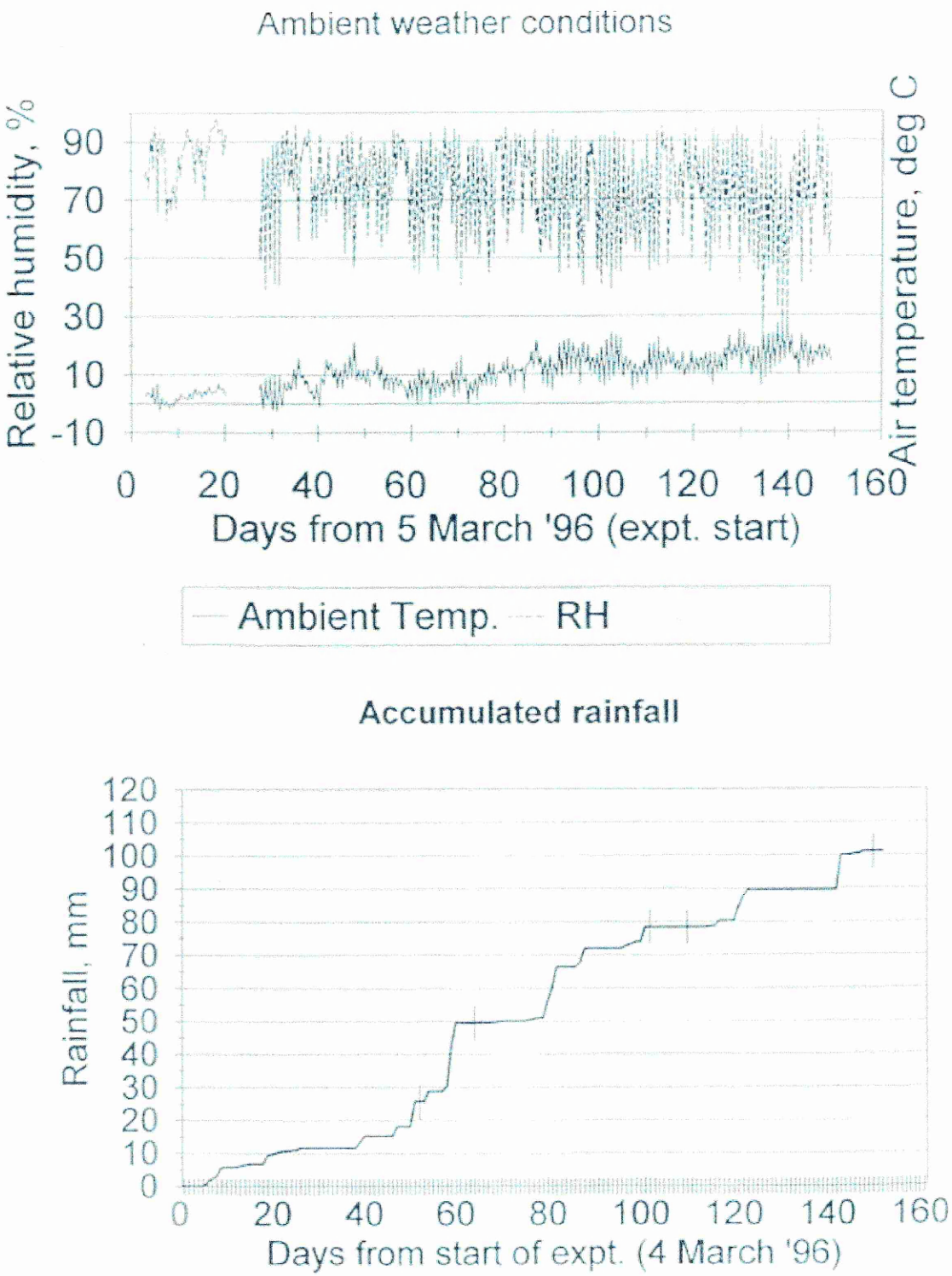


Figure 3.33 Ambient weather conditions and accumulated rainfall data during the 150 day storage period.

3.4.3 Investigation of various treatments to improve straw quality during storage (September 1996-February 1997) (NS4)

A more refined experiment was carried out based on the previous experiments. The experiment used straw from one cultivar (Ribband) and was carried out at only two moisture contents (15 % and 23 %). All of the treatments were carried out prior to baling of freshly harvested straw. Samples were collected after 150 days storage, using the coring device as described previously in Section 2.8.3.

Figure 3.34 showed that treatment with biocide reduced the mean total fungal populations when compared to the untreated controls. The biocide sprayed into straw nets and adjusted to 15 % moisture content had 22 x 10^2 CFU g^{-1} straw whereas, when biocide was not added to the wads of straw in nets 10^4 CFU g^{-1} straw were isolated. Nutri-Shield® had the same number of colonies isolated as the untreated controls. However, 25 % of these colonies were *A.fumigatus*. Chopped straw in nets had the lowest fungal populations at both moisture contents. However, at 15 % moisture content 3 x 10^2 CFU g^{-1} straw were *A.fumigatus*. Because some drying of the bales occurred during the storage period, in some instances the mean total fungal population were higher for straw treatments at 15 %, than 23 % moisture content.

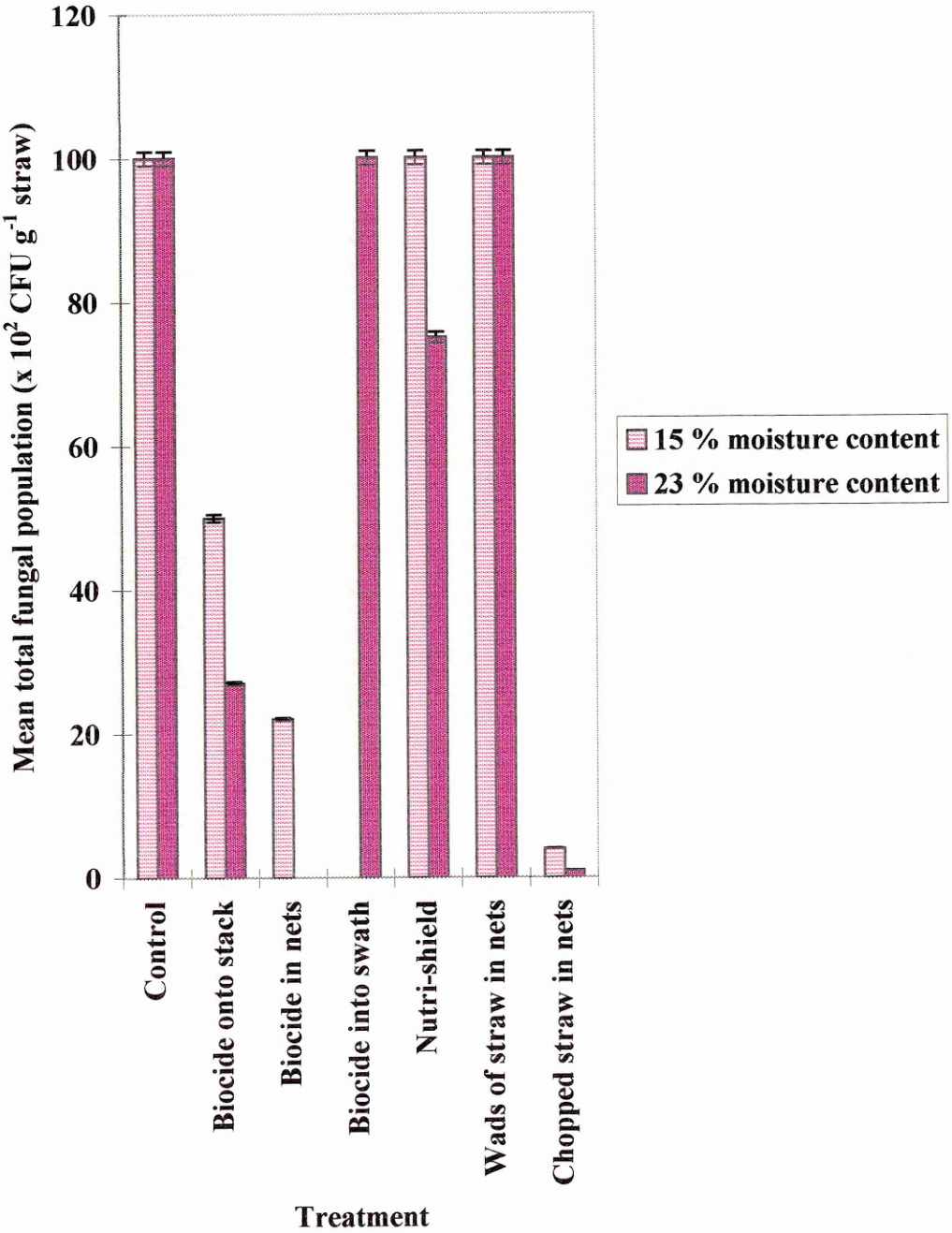


Figure 3.34 The effect of different treatments on fungal populations isolated from the edge samples from top bales of straw after 150 days storage at two different moisture contents (cv.Ribband).

Generally, the total fungal population from core samples (Figure 3.35) were less than the edge samples (Figure 3.34) regardless of treatment or moisture content. Biocide in nets, wads of straw in nets and chopped straw in nets were all effective treatments for controlling microbial growth when compared to control samples. As with the edge samples, the chopped straw in nets had the lowest number of colonies isolated, but at 23 % moisture content the only species isolated was *A.fumigatus*. At 15 % moisture content Nutri-Shield®-treated straw had the largest mean total fungal populations when compared to the other treatments, and 50 % of these were *A.fumigatus*. Nutri-Shield® appeared to promote the growth of *A.fumigatus*, with this trend also seen in the edge samples.

Figure 3.36 showed there were no clear trends for the effect of different treatments on NCP content of edge samples of straw from top bales during storage. The biocide applied into swath treatment decreased NCP content when compared to controls indicating that some degradation has occurred. All of the treatments at 15 % moisture content had greater NCP contents than the controls, the exception was biocide treatment onto the stack. This showed that these treatments were maintaining the NCP content and that the straw was not being degraded.

Generally, in the core samples straw adjusted to 23 % moisture content had higher NCP contents than straw adjusted to 15 % regardless of treatment (Figure 3.37). All of the treatments had lower NCP contents than the control at 23 % moisture content, indicating that some degradation has occurred. The chopped straw in nets had higher NCP contents than the other treatments, and very similar levels to the controls.

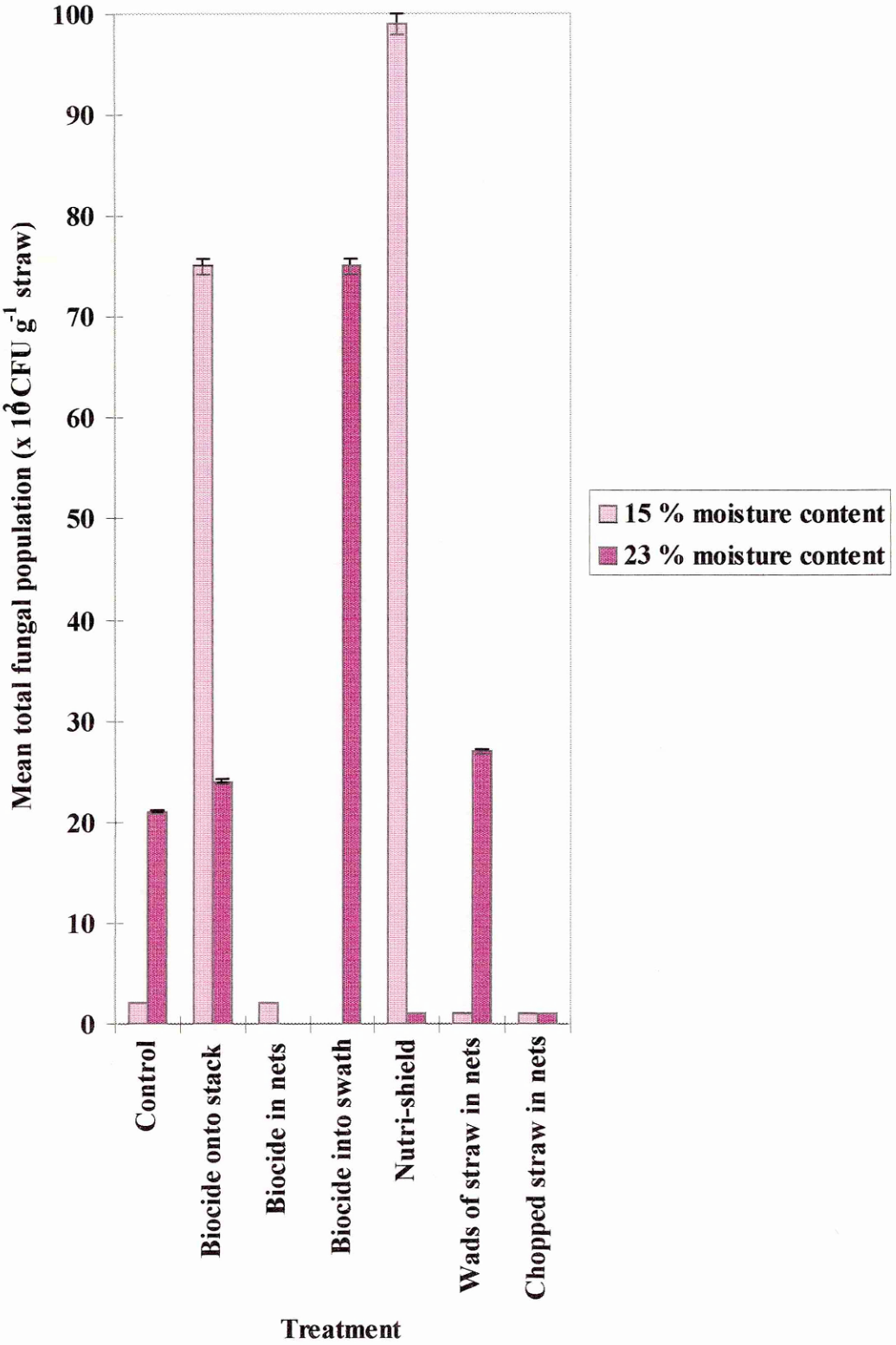


Figure 3.35 The effect of different treatments on fungal populations isolated from the core samples from top bales of straw after 150 days storage at two different moisture contents (cv.Ribband).

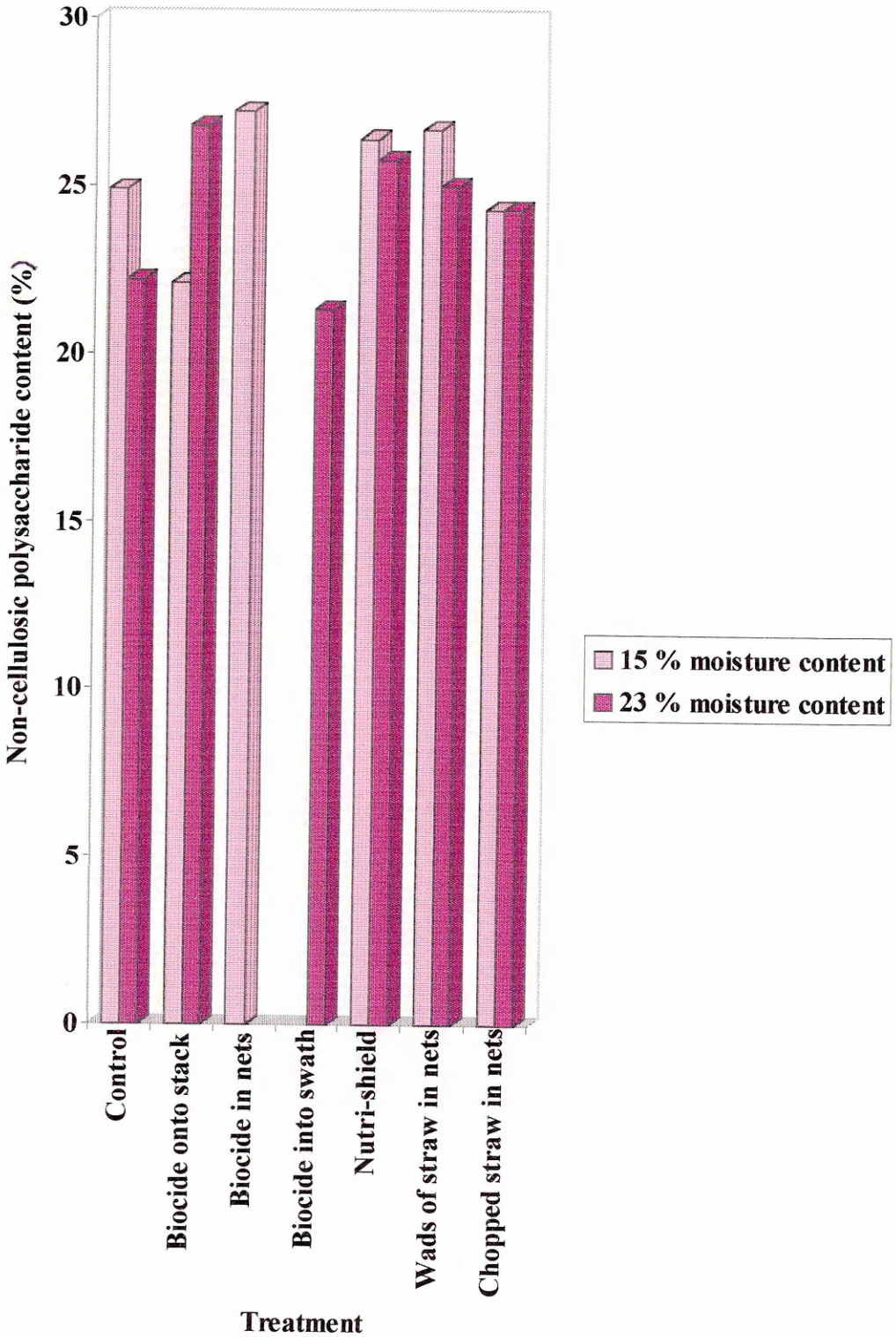


Figure 3.36 The effect of different treatments on non-cellulosic polysaccharide content of edge samples of straw from top bales during storage (cv.Ribband).

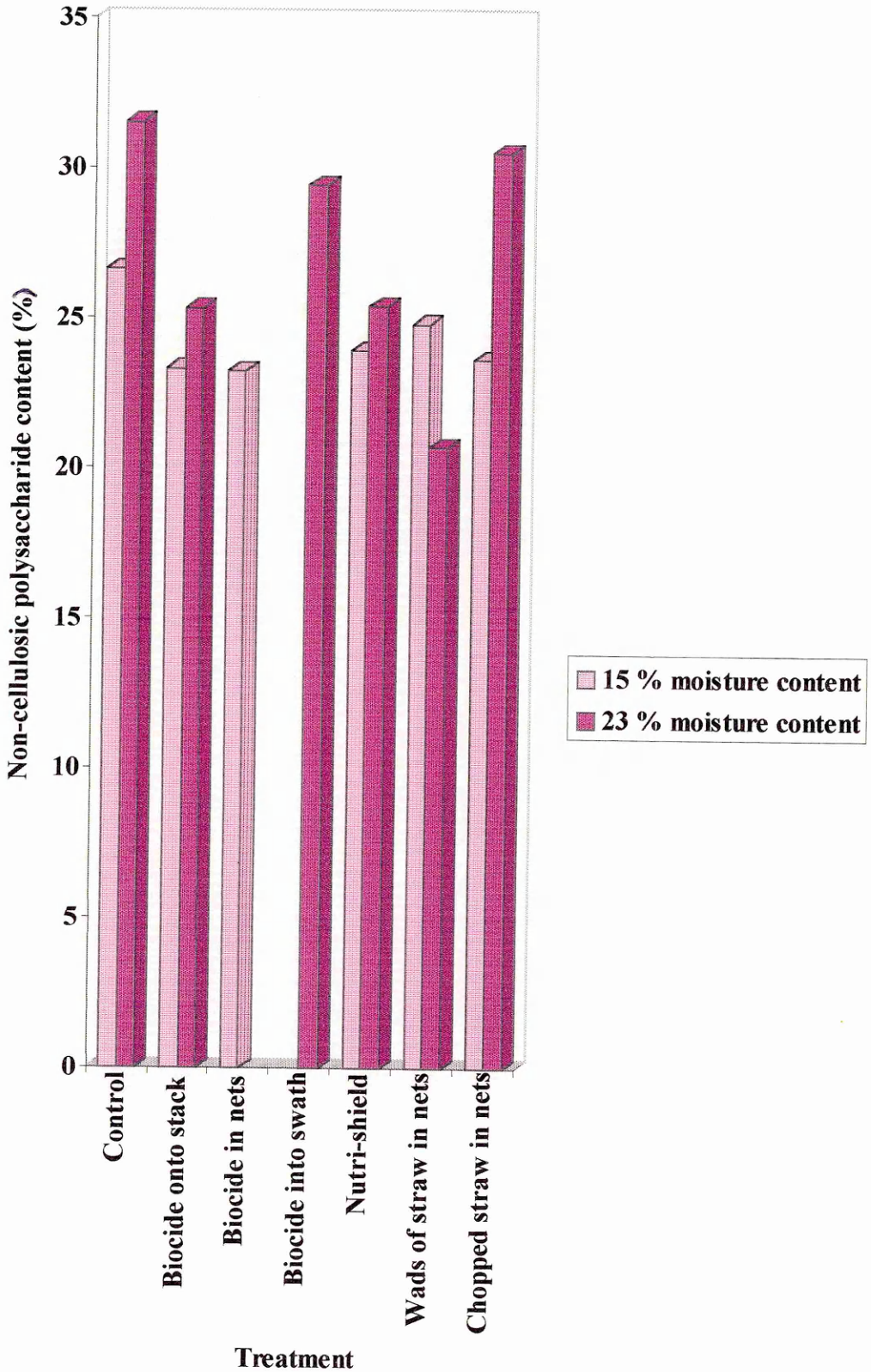


Figure 3.37 The effect of different treatments on non-cellulosic polysaccharide content of core samples of straw from top bales during storage (cv.Ribband).

In the middle bales the edges of the untreated controls had the lowest populations, with the biocide-treated cores having the lowest populations. In the bottom bales biocide had little effect, either at the bale edge or in the core. The Nutri-Shield® treatments were variable with effects being more pronounced in the higher moisture content bales (23 %).

Figure 3.38 showed that the treatments with the biocide sprayed onto the top bale, the biocide sprayed onto the wads of straw in nets, and the Nutri-Shield® treatment, all had poorer lignin content, while other treatments; the wads of straw in nets and the chopped straw in nets were equivalent or better. This trend was seen at both moisture contents.

Similar trends were observed in core samples (Figure 3.39) as were found in the edge samples (Figure 3.38). The exception was wads of straw in nets at 23 % moisture content, where slight increases in apparent lignin content were obtained when compared to the controls, showing that some degradation had occurred with this treatment. The Nutri-Shield® treatment at 15 % moisture content increased the apparent lignin content by 5 % when compared to the control sample. This increase was significant and this treatment showed the greatest degradation of the straw when compared to the other treatments.

Assessment of these changes using DRIFT spectroscopy gave similar results. Samples undergoing no or low levels of degradation showed very little difference in their DRIFT spectra while those undergoing significant degradation, as determined by the increase in apparent lignin content, also gave increased absorbances at 1595 and 1510 cm^{-1}, the main absorbances associated with lignin.

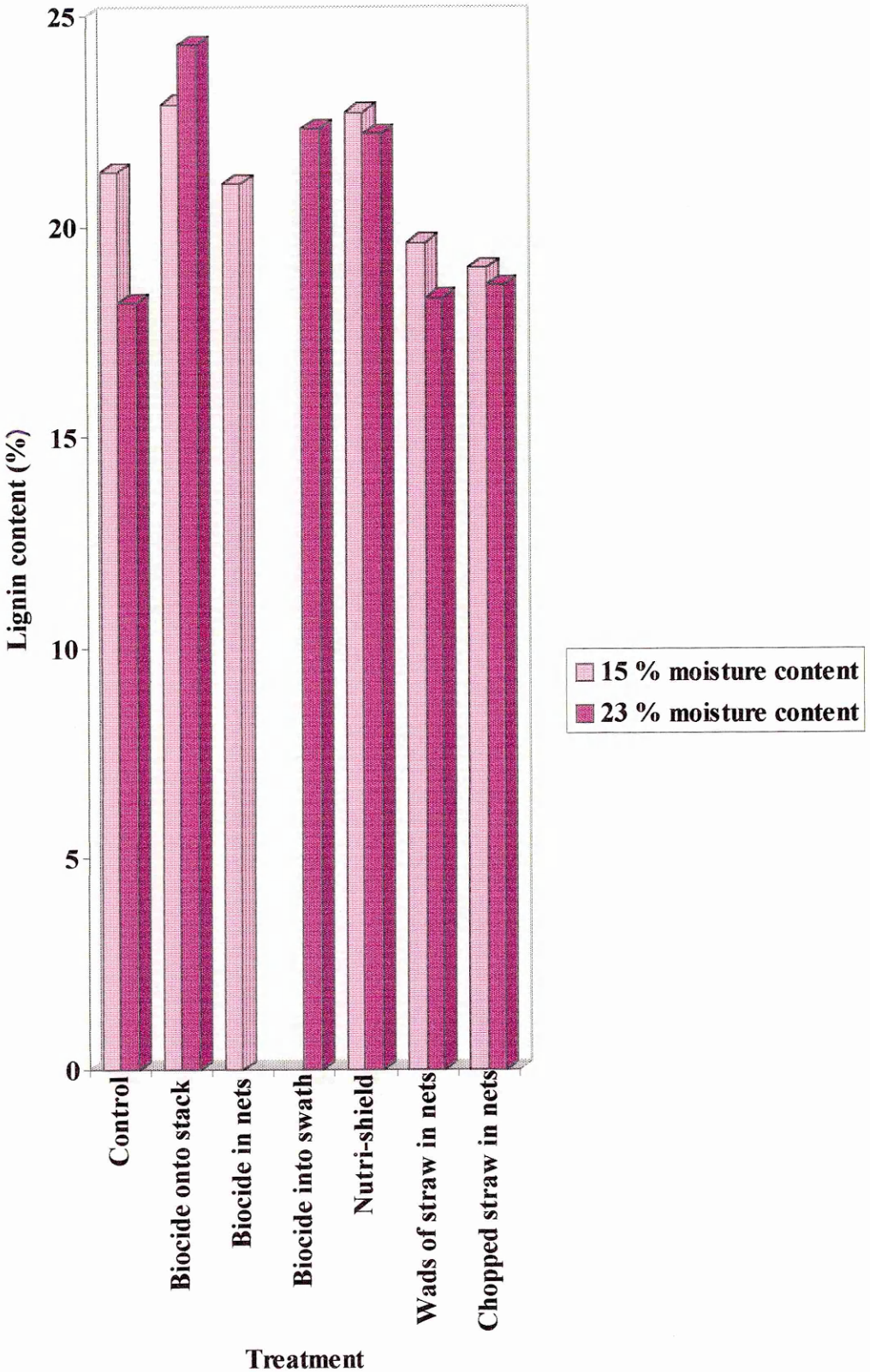


Figure 3.38 The effect of different treatments on lignin content of edge samples of straw from top bales during storage (cv.Ribband).

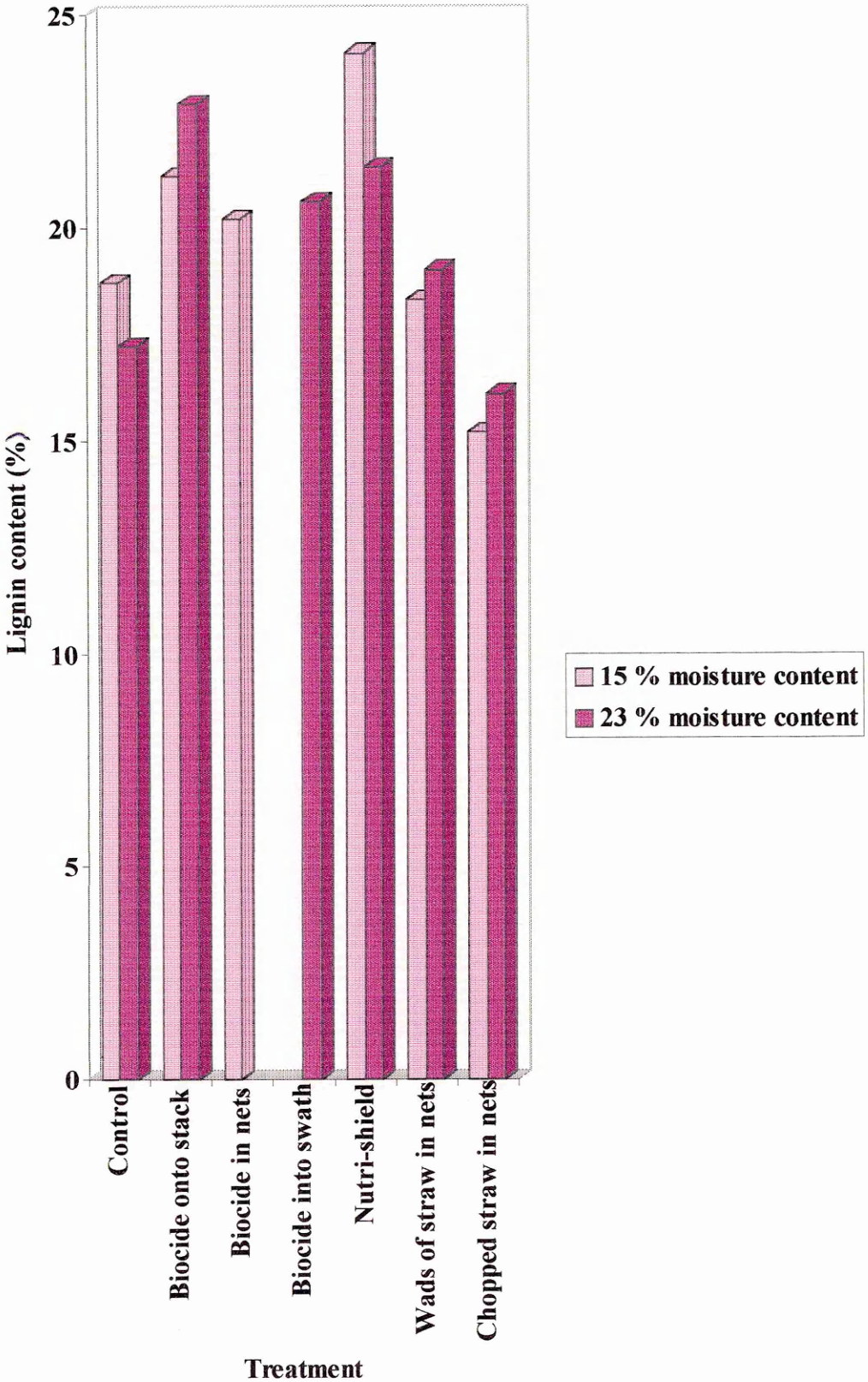


Figure 3.39 The effect of different treatments on lignin content of core samples of straw from top bales during storage (cv.Ribband).

Recordings of bale temperatures and ambient temperatures were made. In this experiment all of the top and middle bale temperatures were monitored. Figure 3.40 showed there were four obvious occurrences of water ingress into the top 200 mm of the top control bale, with penetration to the middle of the second bale down, after a very wet three day period at about 100 days storage when 54 mm of rain fell onto the bales. The bale temperature data during the storage period confirmed the occurrence of short durations of high temperature periods both soon after baling wet straw, and at the ingress of rainwater during storage. They indicated that 'wet at baling' bales (23 %) heat to approximately 50^0C within a few days. Very good correlations were observed between the incidents of heavy rainfall and temperature increases. On day 20 there was approximately 15 mm of rain when the top bales showed a marked increase to about 40^0C signifying water ingress and microbial activity.

Figure 3.41 showed the temperature profiles of wads of straw in nets at 15 and 23 % moisture contents. At both moisture contents when the straw wads were placed horizontally on the top layer bales and retained by a net the rain was absorbed by the straw and then evaporated off before the straw became saturated and released water down to the top bale. The wads on top bales only experienced water ingress (temperature increase) on the occasion of very heavy rainfall around day 100, and then moisture only penetrated into the top bale. No temperature increase was recorded in the middle bale during the entire storage period.

Figure 3.42 showed the ambient temperature and rainfall. These recordings correlate well with the ingress of water and temperature increases in the bales in this experiment.

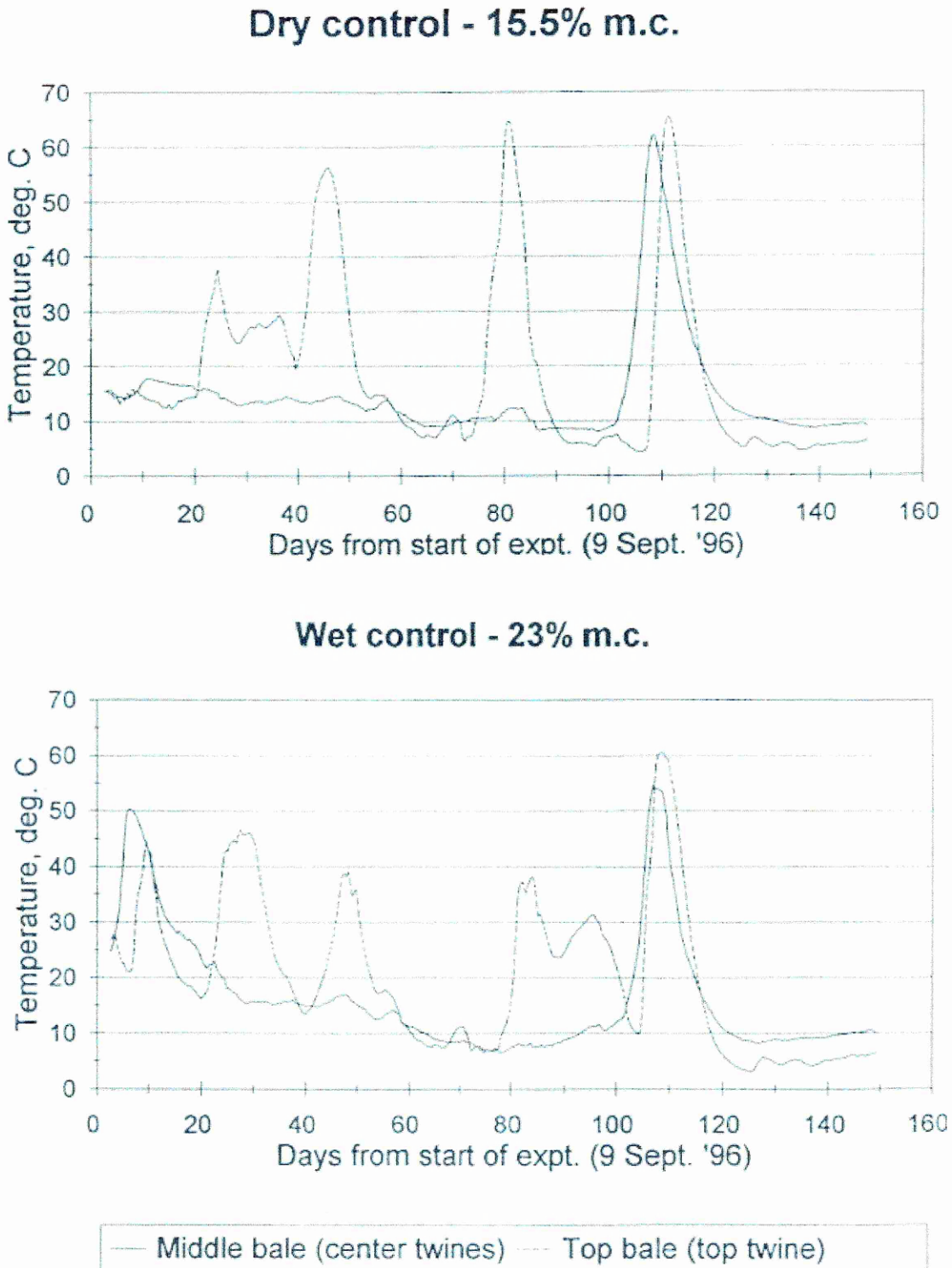


Figure 3.40 Temperature profiles of dry control (15 %) and wet control (23 % moisture content) during storage.

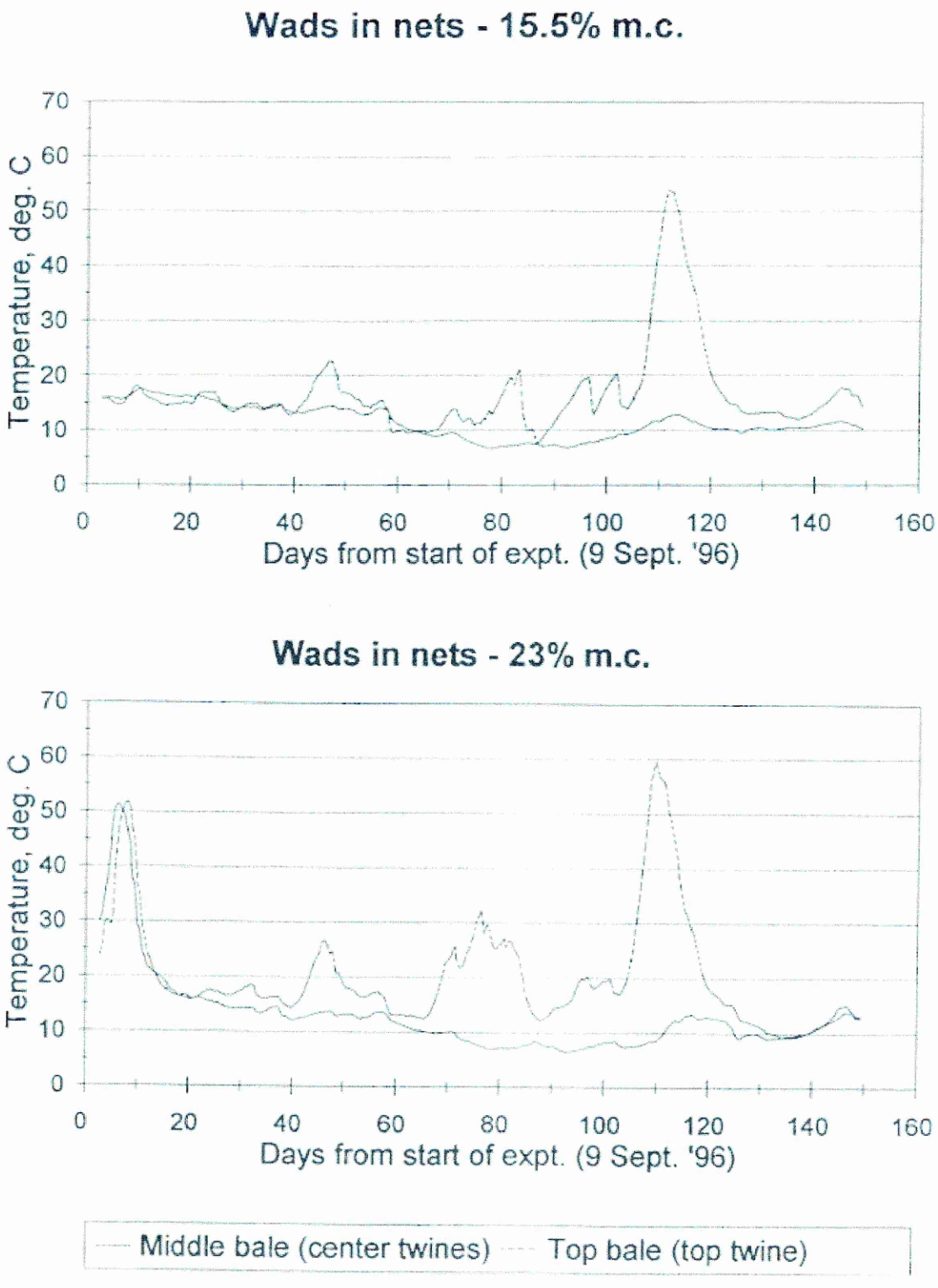


Figure 3.41 Temperature profiles of wads of straw in nets under different moisture contents (15 % and 23 %) during storage.

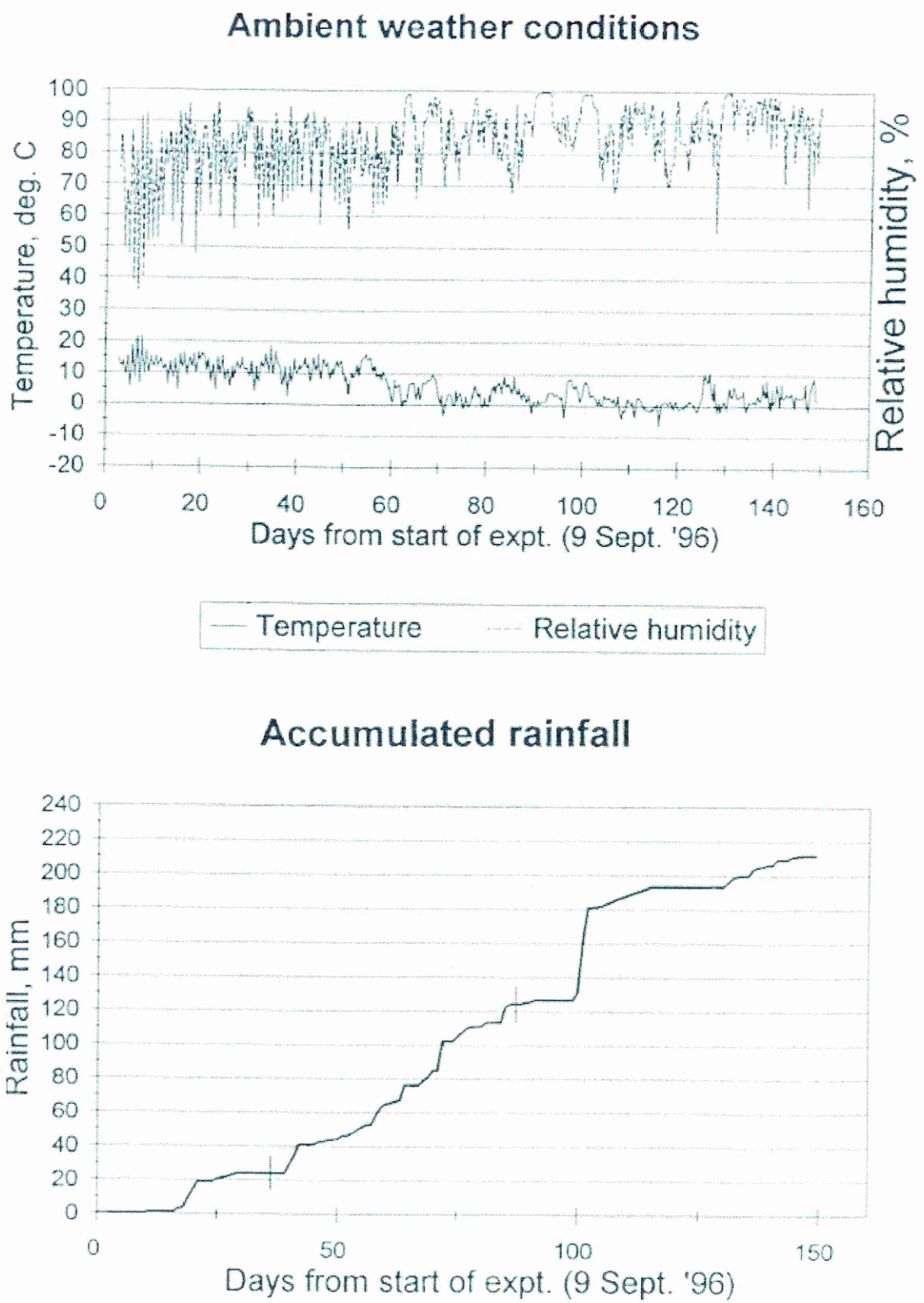


Figure 3.42 Ambient weather conditions and accumulated rainfall data during storage period.

CHAPTER 4

DISCUSSION

4.1 THE EFFECT OF ENVIRONMENTAL FACTORS ON MYCOFLORA, SUCCESSION AND RESPIRATORY ACTIVITY ON WHEAT STRAW

4.1.1 The effect of environmental factors on mycoflora of wheat straw

In previous research about 100 different fungal species have been isolated from wheat straw (Lacey, 1968; Moubasher *et al.*, 1982 a,b; Pelhate and Agosin, 1985). As with cereal grains, the primary mycoflora can be divided into field fungi (e.g. *A.alternata* and *E.nigrum*), the intermediate fungi (*F.culmorum*, *C.cladosporioides* and yeasts), which occur mostly before harvest, and the storage fungi (*Aspergillus* and *Penicillium* spp.) which are sporadic at harvest and only develop later under favourable conditions. Under damp conditions, the mycoflora develops in a similar way to that in hay (Pelhate and Agosin, 1985).

In this study the same range of species were isolated from the two cultivars used (cv. Beaver and Ribband), but the dominance of individual species varied between the two cultivars. This could be due to differing agronomic and nutritional treatments and climatic conditions during crop growth of the two cultivars. This may partially influence the mycoflora on the straw isolated from the cultivars. Previous studies have shown that cultivar may be one of the factors responsible for variations in fungal populations (Magan, 1988a).

C.cladosporioides and *F.culmorum* were present externally on a greater percentage of straw segments than the internal surface when direct plated. *F.culmorum* and *C.cladosporioides*, both intermediate fungi, would probably have colonised the straw pre-harvest and their spores would be present on the surface of

the straw. Therefore it was not surprising that these species were dominant on the external surface. The storage conditions may not have been conducive for mycelial growth into the internal structure of the straw, so these species were in lower concentration when compared to the external surface.

As temperature was increased from 10^0C to 30^0C, the number of different species and their dominance changed. Very little growth occurred at 10^0C with a maximum at 25^0C. At 30^0C more thermotolerant species, such as *A.fumigatus*, *Absidia* spp. and *A.flavus* were present. This indicates that fungal colonisation and growth is temperature dependent. Consequently, deterioration of the straw during storage would also be temperature dependent. This trend has been shown by previous research on other agricultural materials, such as hay (Lacey, 1980b), sugar cane bagasse (Lacey, 1974), and wheat straw (Magan, 1988a,b).

As a_w was increased, making water more freely available, a wider range of species was isolated and dominance changed. This trend agrees with previous research (Magan and Lynch, 1986; Magan, 1988b). At 0.95 a_w, the greatest range of species were isolated, this was due to the fact that the amount of available water to the microbial cells and enzymes determines their activity and was not limited. In this study, the radial growth of *F.culmorum* at 0.95 a_w was 4.5 mm day^{-1} at 10^0C, which was higher than previous findings by Magan and Lacey (1984a), who found that isolates from grain had a radial growth of 1 mm day^{-1}. However, they excluded early exponential and late sub-optimal growth, and the isolate was grown on a nutritionally weaker wheat extract agar, which may explain the differences.

In this study, the effect of pH on microbial colonisation and growth was investigated. The dominance of species varied with pH. For straw plated on pH-adjusted media and incubated at 25^{0}C, at pH 4.4 the dominant species was *A.niger* whereas, at pH 6.4 the species was *C.cladosporioides*. Most previous studies have not considered pH and its effects on microbial colonisation and growth on straw. Thus no direct comparisons can be made.

Generally, in this study *F.culmorum* was isolated over the pH range 4.4-6.4. As *F.culmorum* was isolated over this pH range, which is the optimum for cellulase activity, it may have degraded the straw during this storage period, although further tests would need to be carried out to confirm this. Therefore, the pH of any treatment may be important if degradation of straw by cellulolytic fungi is to be avoided. A previous study by Forbes and Dickinson (1977) investigated the effect of temperature, pH and nitrogen on cellulolytic activity of *F.avenaceum*. They found that the pH range for optimum cellulolysis by *F.avenaceum* was particularly narrow (pH 4.4-5.4), contrasting with the very similar levels of cellulolytic activity throughout the range pH 3.7-8.6 shown by an unnamed *Fusarium* spp. used by Sharp and Eggins (1970). *F.avenaceum* does however, conform to the general pattern for fungal cellulases (Mandels and Reese, 1960), which are active between pH 3.5 and 7.0 and show optimum activity at pH 4.0-5.5.

During any storage regime, the interactions between a_w, temperature and pH are important considerations for preventing fungal colonisation and growth. In this study for the first time, all three environmental factors and their interactions were investigated. On 0.95 a_w-adjusted media with a pH of 4.4 at 25^{0}C, *F.culmorum*, *R.pusillus* and *T.harzianum* achieved maximum growth over 7 days, indicating that

these environmental conditions were conducive for these species. The interactions of these environmental conditions showed that for each fungus as the a_w increased, the rate of radial growth increased slightly with temperature.

4.1.2 Effect of environmental factors on fungal respiration on wheat straw

Respiration has been used to measure metabolic activity and dry matter losses in stored produce for a long period of time by many researchers. At present there are a variety of methods to measure respiration. The technique employed in this study was the use of an electrolytic respirometer system, designed by Tribe and Maynard (1989). This method was previously successfully used by Hamer *et al.* (1991) and Lacey *et al.* (1994), for studying the respiration of cereal grains at different a_w levels and temperatures. It was shown in their studies that efficient and accurate measurements of respiration of grain samples and the colonising mycoflora could be achieved, with minimal disturbance and destructive sampling required during the monitoring period. The design of the respirometer system was modified in this study, as previously explained in Section 2.5.1. The reason for the modification was to enable a greater volume of chopped straw to be used in each experiment.

Respiration of naturally contaminated wheat straw was determined over a range of environmental conditions. There was a steady increase in respiratory activity as storage temperature was increased. This was probably due to the fact that fungal activity was temperature dependent, as was shown in the previous in vitro experiments in this study. This was also observed by Hamer *et al.* (1991), although they were using grain, which also respired. In the present study at the highest temperature investigated (30^0C) there was almost a linear increase, whereas at the

lowest temperature (10^0C) a lag phase occurred prior to fungal respiration. Fungal colonisation and respiration on straw was significantly influenced by temperature and this indicated that it is an important environmental factor influencing respiratory activity of fungi and deterioration of cereal straw.

There was also a steady increase in respiratory activity as a_w increased. At 0.95 a_w, oxygen consumption (respiration) at each temperature was almost 50 % greater than at corresponding temperatures at low a_w (0.75), when very little fungal activity occurred. Fungal colonisation and respiratory activity on straw was thus significantly influenced by a_w. These results confirm the in vitro studies, which showed that fungal growth was dependent on the prevailing water availability. Respiratory activity at 0.98 a_w was almost three times that at 0.75 a_w at 25^0C. This may be due to the greater number of species that could colonise the straw rapidly at this high a_w. A temperature of 25^0C is often the optimum for fungal growth of many mesophilic straw fungi. For many of the a_w levels tested, a lag phase occurred of up to 24 hours, when there was little respiratory activity. Previous studies by Hamer (1994) also found that in grain, with <0.90 a_w, respiration increased in a non-linear fashion with time, with an initial lag phase followed by subsequent more rapid fungal activity. However, at a_w >0.90 a_w, respiration increased in a linear fashion with time, in grain, as observed in this study with straw.

Generally, in this study, respiratory activity thus increased with temperature x a_w interactions. These factors are critical in influencing the rate of fungal colonisation and deterioration in straw quality. Previous research by Hamer *et al.* (1991) and Lacey *et al.* (1993) also found that respiration of grain and its

mycoflora were dependent on temperature x a_w interactions. However, the respiration measured in this study was due solely to the fungal activity of naturally occurring mycoflora on straw, which is a dead material. Whereas, that measured by their studies was the combined respiration of the grain and the naturally occurring mycoflora on the grain.

The mycoflora present on the stored straw was enumerated at the end of each experiment to determine which species were present, and dominant under various temperature x a_w storage regimes. Fungal populations varied with a_w, as previously found in studies on the succession on grain; straw in soil and stored straw (Magan and Lacey, 1984 b; Harper and Lynch, 1985; Magan and Lynch, 1986; Magan, 1988a;).

The xerophilic species, *E.amstelodami* was isolated over the widest a_w range (0.75-0.90) when compared to the other species. Previous studies by Hamer (1994) showed similar results on wheat grain. She isolated *Eurotium* spp. from grain adjusted to 0.80-0.95 a_w after storage for up to 14 days in respiratory chambers. The only other species isolated over the whole a_w range were other *Aspergillus* spp. In the present study at high a_w (0.98) *Aspergillus* spp. were only present at the highest temperature tested (30^0C), indicating that they were thermotolerant. Hamer (1994) also isolated *Penicillium* spp. and *Rhizomucor* sp. at 30^0C. However, the highest a_w tested was 0.95 a_w.

F.culmorum was the only species present over the whole temperature range in this study, indicating a wide adaptability to environmental conditions. This may be explained by the fact that *F.culmorum* is a good competitive saprophyte (Sivan

and Chet, 1989). It may have successfully competed against the other mycoflora present on the straw and become the dominant species. By contrast, *F.culmorum* was only isolated from grain stored at 15^0C at 0.90 and 0.95 a_w on grain (Hamer, 1994).

As dry matter loss (DML) occurs by the utilisation of carbohydrate during fungal respiration, the data may also be used to quantify the deterioration losses. The DML caused by natural mycoflora on straw under various storage conditions was calculated in this study for the first time. As a_w was increased DML increased. This correlated with the fact that respiratory activity also increased with an increase in a_w. Under high a_w (0.90-0.98) as temperature increased from 10-30^0C the amount of DML increased. However, this trend was not seen at the other a_w levels tested. The maximum percentage DML was 3.40 % at 30^0C and 0.98 a_w. This may be due to species such as, *T.harzianum* and *F.culmorum*, which are cellulolytic.

Table 4.1 showed the comparison between deterioration of wheat straw and wheat grain. The DML of wheat straw in this study were after 14 days storage whereas, the DML of grain were after 7 days storage. Generally, deterioration of straw was significantly greater (P <0.05) than in grain (Hamer, 1994). This was due to straw being more susceptible to deterioration due to it being a dead material, whereas, the grain was capable of preventing as much deterioration due to it being a living material.

Table 4.1 Comparison of dry matter losses of grain (Hamer, 1994) and wheat straw.

Material	a_w	DML (%)			
		Temperature (^{0}C)			
		15	20	25	30
Grain	0.90	0.085	0.226	0.436	0.347
	0.95	0.517	0.762	1.21	1.187
Straw	0.90	0.44	0.48	0.56	2.59
	0.95	0.38	0.73	0.74	3.39

In the present study, no visible moulding was observed after 14 days storage. However, Hamer (1994) found that apart from samples stored at 15^0C and 0.90 a_w, all of the other samples were visibly mouldy. This difference was due to grain being a living, respiring material whereas straw was dead.

To compliment the DML calculated from the respiration data, samples of straw were analysed for changes in lignin and NCP. In order of availability, any carbohydrate derived residual cell contents will be degraded first, followed by degradation of the carbohydrate components of the cell wall or fibre. Degradation of NCP usually begins before degradation of cellulose starts but degradation of both will then progress in parallel. Degradation of the NCPs is not complete before degradation of cellulose begins. Natural degradation of lignin will only occur when extensive degradation of the carbohydrate components (cellulose and NCP) has occurred. Degradation of lignin is usually considered as a secondary process which is only initiated when the more readily degradable fraction of the carbohydrate sources are used up. The lignin content, to a first approximation, can be considered as an inert marker and used to assess the loss of other components. Of course, with other constituents being lost by degradation, the apparent lignin content will rise. This is not due to synthesis of lignin: merely the mathematical result of the lignin being in a higher proportion of the residual straw sample.

Only the results of the lignin determination and the DRIFT spectra were presented, in this aspect of the study. At 10^0C there was no significant change in lignin content irrespective of a_w while at 15^0C, there was a slight reduction in dry conditions (0.75 a_w) but this increased as a_w increased. The greatest increase was observed at 20^0C, when apparent lignin content rose with increasing a_w and once

0.90 a_w had been reached remained at 140 % of the starting value. This indicates significant loss of carbohydrate components, assuming no loss of true lignin. Results at 25^0C and 30^0C were less clear. At lower a_w there was an increase in apparent lignin content but not as high as at 20^0C. A possible explanation is that at these higher temperatures and a_w levels, degradation of true lignin occurred but this could not be confirmed.

The DRIFT spectra allowed structural changes in cellulose to be visualised. Cellulose undergoes changes in its secondary and tertiary structure, often before loss of dry matter is evident. These changes involve the molecular order of the cellulose and affect the ability to spin long fibres from cellulosic substrates such as flax or cotton, and the alignment of short fibres from cellulosic substrates such as wood, in the manufacture of paper. The DRIFT spectra confirmed that degradation of carbohydrate was occurring.

The contribution of individual species to straw deterioration over a range of a_w and temperature interactions was also investigated in this study. *F.culmorum* had the greatest temporal cumulative oxygen consumption (respiratory activity) when grown in pure culture on sterile straw. Under the same conditions *C.cladosporioides* had the lowest oxygen consumption. Each species had relatively different respiratory rates and the results showed that interactions with other species can influence overall respiratory activity. Each species increased their respiratory activity at 25^0C when compared to 10^0C, the same trend was seen for respiration of natural mycoflora on straw. *F.culmorum* nearly doubled its respiratory activity at 25^0C when compared to 10^0C. This showed that temperature

and a_w influenced the respiration of individual species and were important environmental factors.

The only previous research similar to this experiment was by Hamer (1994). She irradiated grain and re-inoculated it with *E.amstelodami* or *P.aurantiogriseum* or a mixture of the two species, onto grain adjusted to 0.85 a_w or 0.90 a_w at 20^0C. *E.amstelodami* showed the least respiratory activity at 0.90 a_w after 14 days storage when compared to *P.aurantiogriseum* and a mixture of the species. By contrast, in the present study, *E.amstelodami* had a greater respiratory activity at 0.95 a_w and 0.98 a_w than *P.aurantiogriseum*, but the latter species had greater respiratory activity at 0.85 a_w. However, the present study compared a wider range of species than that on grain by Hamer (1994).

Dry matter losses on straw were determined for each individual species, so that their individual effect on quality could be compared. Each individual species caused very different DML on straw. Generally, as a_w increased DML increased for all of the temperatures tested. The maximum DML was 11.93 %, caused by *F.culmorum* at 0.98 a_w and 25^0C. In Hamer's study (1994), *E.amstelodami* caused the least DML, and it also had the lowest respiratory activity. The results from the present study suggests that both antagonistic and synergistic effects must occur in naturally contaminated straw influencing the dry matter losses which occur at particular temperature x a_w storage regimes.

4.1.3 The effect of a_w and temperature on fungal succession and dry matter losses of wheat straw

Storage experiments were undertaken over 6 months to determine the succession of fungi on straw under a range of temperature x a_w interactions. The DML of straw under these different storage regimes were also calculated. Generally, total fungal populations increased when temperature was increased during the storage period. Futhermore, a wider range of species were also investigated. This trend was observed at each a_w investigated. Generally, as a_w increased the fungal population increased. This trend has previously been observed in many studies. The dominant species isolated at all a_w levels over the 6 month storage was *P.aurantiogriseum*. This species predominated over the storage period at 15^0C, and at all a_w levels tested.

In addition, *E.nigrum* was isolated after 2 months and 6 months at 0.98 a_w and 0.90 a_w respectively. However, this species was not present at 10^0C. After 6 months *T.harzianum* was isolated at 0.95 a_w and 0.98 a_w. These observations show how changes in dominance and succession occur with storage and environmental conditions. A previous study by Magan and Lynch (1986) also found that *T.harzianum* colonised straw pieces best at high water potential (-0.7 MPa, ≡0.995 a_w), but was more sensitive to drier conditions. Previous studies have shown that *Trichoderma* spp. can reduce pathogen populations in dead plant tissue (Mew and Tosales, 1985), although this was in rice straw and was not observed in this study. The variety of mechanisms by which *Trichoderma* spp. can antagonise pathogens makes them candidates for the control of a whole range of straw-inhabiting pathogenic fungi. A previous study by Fernandez (1992) found that *T.harzianum*

when inoculated onto wheat straw at harvest resulted in a reduction in the incidence of *Fusarium* spp.

Previous studies on the fungal succession on cereal residues have mainly concentrated on other crops, such as wheat compost (Chang and Hudson, 1967), sugar bagasse (Sandhu and Sidhu, 1980) and grains (Clarke and Hill, 1981). Since straw is a waste product its spoilage has received less attention when compared to research on the mycoflora of hay and grains. As straw is often incorporated into soil, a variety of studies have previously been undertaken on the colonisation and decomposition of straw in agricultural soils (Harper and Lynch, 1981; Harper and Lynch, 1985; Broder and Wagner, 1988; Magan *et al.*, 1989; Robinson *et al.*, 1994).

The only previous study that has investigated fungal colonisation and decomposition of wheat straw was by Magan (1988a). In his study the environmental conditions were limited to 15^0C and 25^0C and the water potentials -0.7 MPa (≡0.995 a_w), -2.8 MPa (≡0.98 a_w) and -7 MPa (≡0.95 a_w) for 12 weeks. In the present study a wider range of temperatures and a_w levels were tested over a longer, more realistic storage period. The predominantly isolated species in this study was *P.aurantiogriseum*, over the whole range of a_w and temperatures tested. Magan (1988a) also found that *Penicillium* spp. were predominantly isolated at 0.95 a_w. However, at 0.98 a_w *Penicillium* spp., *Rhizomucor* spp., *Rhizopus*, *Trichothecium* and occasionally *Fusarium* spp. were present at 15^0C storage. These species were not often isolated in the present study. This may be partially due to the use of different cultivars in these two studies, which can influence the mycoflora found (Magan, 1988a). At 25^0C the patterns of colonisation were

different when compared to previous work (Magan, 1988a). He mainly isolated *Aspergillus* spp. at 0.95 a_w, with *Penicillium* spp. isolated from 72 % of straw segments at 25^{0}C after 2 months storage. In this study, *P.aurantiogriseum* was isolated from 58 % of straw segments. In the present work it was concluded that a_w, temperature and storage period all affected the dominance and succession of species on straw.

Previous research in other climatic regions have isolated different dominant species from cereal residues. In Bulgaria, *A.alternata* was isolated from all straw samples, both at harvest and after 4-5 months storage. Other common species included *A.fumigatus*, followed by *Rhizomucor hiemalis* and *Penicillium* spp. *A.flavus*, *F.moniliforme*, *Chaetomium comatum*, *Rhizopus stolonifer*, *Scopulariopsis brevicaulis*, *C.linicola* and *T.viride* while *Stachybotrys atra* occurred less frequently (Alexandrov, 1986). In Pakistan, moulding occurred in 30 % of wheat straw samples, with *Aspergillus*, *Penicillium*, *Rhizomucor* and *Alternaria* spp. predominant (Mir and Ali, 1990). In India, most fungi from decaying straw and other substrates were *Aspergillus* spp., but *Acremonium* spp., *Curvularia lunata*, *Gliocladium fimbriatum* and *Monilia* spp. were also isolated (Singh and Singh, 1991).

Growth of fungi on straw results in its degradation and loss of dry matter. In this study for each of the temperatures, as a_w increased, the percentage DML increased over the storage period. This finding agrees with previous research (Magan, 1988a). The effect of temperature on DML was variable in this study. However, Magan (1988a) found that DML of wheat straw during the exponential period was greater at 25^{0}C than at 15^{0}C with the greatest loss at 0.995 a_w. Similar DML were

obtained in experiments with barley straw. Previous studies on rapeseed found that DML over a 4 week period were also greater at 25^{0}C than at 15^{0}C, regardless of a_w.

Sain and Broadbent (1975) also found the rate and extent of rice straw decomposition were found to increase with the lag period decreasing with increasing relative humidity and temperature. In this study, DML of straw was much greater than previous findings. At 15^{0}C storage, DML was 17 % after 4 months storage at 0.95 a_w, compared to 7 % DML after 3 months storage (Magan, 1988a). Only DML of straw after storage at 25^{0}C and 0.98 a_w were similar to those obtained by Magan (1988a), being 29 % DML and 22 % DML respectively. A possible explanation may be that the cultivar used in this study was more susceptible to decomposition and there was a greater proportion of cellulolytic fungi colonising the straw.

Studies of degradation of straw have often been made in the soil environment. For instance, decomposition of intact lengths of straw inoculated , either together or in succession, with pairs of fungi, chosen from *F.culmorum*, *T.viride*, *C.globosum* and four lignin-degrading basidiomycetes and a *Typhula* sp., was generally characteristic of the isolate that was the more effective decomposer in pure culture. However, some combinations showed evidence of interactions which usually increased, but sometimes decreased, the rate of straw decay compared to pure cultures of the more effective decomposer (Bowen, 1990). Previous research on DML caused by individual species have generally shown much higher DML when compared to this study. Chawla and Kundu (1985a,b) found that *A.alternata* caused 50.8 % loss in pure culture. This DML was over twice that found in the

present study. This indicates that some competition and possibly suppression of species was occurring in the straw during storage in this study, and as a result DML was lower. The reason for DML of straw is enzyme activity in the substrate and enzymes decomposing cellulose, hemicellulose, xylan, lignin and other components by straw decomposing fungi. Each species has different degrading capabilities, and their dominance on the straw, the environmental conditions and storage period will all reflect the amount of DML.

4.2 USE OF BIOCIDES TO CONTROL FUNGAL ACTIVITY ON STRAW

4.2.1 In vitro studies on efficacy of biocides on fungal activity

All of the biocide-adjusted media tested reduced the natural mycoflora on straw segments, when compared to the controls regardless of concentration. All of the biocides appeared to be fungicidal. Lastil 40, Adesol 20 and Busan 881 at 100 ppm concentration inhibited fungal growth from straw segments in vitro studies over the temperature range 10-30^{0}C. Generally, as temperature of incubation was increased the isolation of fungi increased, regardless of concentration. Of the biocides tested, Lastil 40 had the greatest efficacy, reducing fungal populations significantly (P<0.05) at all concentrations.

Most studies which investigated the use of preservatives and fungicides to reduce total fungal populations have involved applications onto hay (Lord *et al.*, 1981; Magan and Lacey, 1986 a,b) or on stored wheat grain (Magan, 1993). Surprisingly, there have been very few studies on reducing the natural mycoflora of straw using fungicides. This may be due to the low economic value of wheat straw

when compared to the more lucrative business of grain storage. Because of the large economic losses that can occur when grain is stored incorrectly, there have been many studies on grain storage regimes and prevention of losses caused by micro-organisms (Magan, 1993; Lacey *et al.*, 1994).

The results in this study cannot be directly compared to other studies as different fungicides, preservatives and agricultural materials were tested. Research by Lord *et al.* (1981) was the only study that included the effect of propionic acid on the natural mycoflora of hay, in addition to the effect on individual fungal species, as carried out in the present study. Non sterilised propionic acid-treated straw was stored in Dewar flasks and although larger concentrations of acid inhibited growth, the organisms were not killed and growth was only delayed. This was partially due to the propionic acid acting as a fungistat, whereas, the biocides tested in this study were all fungicidal.

Previous studies by Magan (1993) using sulphur dioxide (SO_2) to control mould spoilage of grain found that the growth rate of *P.aurantiogriseum* increased from 0.2 to 0.7 mm day^{-1} when grown on MEA adjusted with 100 ppm SO_2 incubated at 15^0C and 25^0C respectively. A similar trend was observed at the other concentrations tested. In the present study all of the biocides inhibited growth of individual fungi tested in vitro at 100 ppm. This was much lower than in other studies. Magan (1993) found that treatment with SO_2 in solution at 500-2000 ppm decreased total fungal populations on grain but did not totally inhibit growth. However, SO_2, like propionic acid-based preservatives act as fungistats not fungicides.

The efficacy of biocides at controlling individual fungal species over a range of temperatures was investigated in this study. Species varied in their sensitivity to the biocides tested. All concentrations (0.8-100 ppm) were to varying extents effective at controlling radial growth when compared to the untreated controls. Lastil 40 was most effective of all of the biocides tested, regardless of temperature. As biocide concentration increased the radial growth rate decreased for each species. However, none of the biocides were as effective at reducing the radial growth of *T.harzianum* and *R.pusillus* when compared to the other species. However, the biocides reduced radial growth of both species by 50 %, when compared to the untreated controls. The species that had the maximum reduction in radial growth was *F.culmorum*, by 66 %. Surprisingly, increasing the biocide concentration did not significantly reduce the radial growth of *T.harzianum* and *R.pusillus*.

Most previous studies have concentrated on the effects of fungicides or fungistats on individual species, particularly *Aspergillus* and *Penicillium* spp.(Lord *et al.*, 1981; Magan and Lacey, 1986 a,b). However, a study by Magan (1993) investigated the effect of SO_2 on other species including, *C.herbarum*, *E.nigrum* and *A.pullulans*. He found that *C.herbarum*, *E.nigrum* and a range of *Penicillium* spp. were tolerant of concentrations of up to 200 ppm. However, in the present study these species were tolerant of only about 80 ppm of Busan 881 in vitro. Busan 881 (0.8 ppm) reduced growth of *E.nigrum* by 50 %, which is a considerably lower concentration than that of SO_2 (50 ppm) required by Magan (1993). Very few studies have however have examined the relationship between a_w x preservative efficacy. Magan and Lacey (1986b) examined the effects in lag

time, growth rates and found that a_w was a significant factor. In this study inhibitory effects varied with a_w x concentration interactions.

4.2.2 Control of fungal respiratory activity on straw using biocides

Previously no attempts have been made to screen biocides at different a_w levels, and by monitoring respiratory activity after application to naturally contaminated straw. Respiratory measurements are an effective method for evaluating efficacy of biocides. In this study biocides were sprayed onto straw at different a_w levels. Respiratory activity at 0.98 a_w on control straw was increased by 50 % when compared to respiration at 0.95 a_w. This demonstrated that respiratory activity was influenced by a_w.

In this study, the efficacy of Busan 881 was slightly diminished with increasing a_w. It was more effective at 0.95 a_w than at 0.98 a_w. This may be due to the increase in the number of species colonising the straw.

Surprisingly, increasing the concentration of Busan 881 did not increase efficacy for the control of fungal activity on the straw (see Figure 3.2.1). A possible explanation may be that the active ingredients in the biocide had a stimulatory effect on the straw mycoflora at the higher concentrations. A similar phenomenon was seen in previous studies by Magan (1993). He found that when SO_2 was used to control fungi in stored grain, *Penicillium* spp. were able to tolerate up to 250 ppm at 25^0C. However, growth was stimulated by intermediate concentrations of 100 ppm at 0.95 a_w.

All of the biocides tested reduced fungal respiration when compared to the controls. The most effective biocide was Lastil 40 (100 ppm), which decreased fungal respiration by 66 % when compared to the controls. This confirmed previous in vitro studies which showed a similar trend.

All of the biocide treatments reduced DML when compared to controls at both a_w's (0.95 and 0.98). For all treatments less dry matter losses were observed in the drier (0.95 a_w) than the wetter straw (0.98 a_w).The most effective treatment, Lastil 40 reduced DML to 0.26 % (at 0.95 a_w) and 0.33 % (at 0.98 a_w) when compared to controls which had a DML of 0.74 % and 0.81 % respectively. This reduction of DML of straw could have important economic implications for people who store straw, provided this method could provide effective control over long storage periods. The efficacy of the biocides against the main spoilage fungi on straw thus lead to further field studies to examine their potential use.

4.2.3 Field Trials

All of the biocides tested in the initial field trials (NS2) significantly decreased the mean total fungal populations on straw when compared to the untreated controls, although their efficacy varied. A successful biocide must have very low mammalian toxicity but wide and long lasting microbial inhibiting properties. The efficacy of biocides depends on effective disturbance of fungal metabolism, and by prevention of sterol synthesis, inhibit cell wall expansion.

Busan 881 was effective at reducing total fungal populations when compared to the control edge and core samples, and was more effective than Adesol 20 but less

effective than Lastil 40. Several groups of biocides disturb the respiration of fungi and all such compounds are powerful inhibitors of spore germination. The biocide Busan 881 belongs to one of the oldest group of biocides, the dithiocarbamates. The dithiocarbamates owe their toxicity to their ability to chelate with certain metal ions, especially copper and can inhibit several enzymes involved in respiratory processes. One of the active ingredients in Busan 881 is potassium N-methyldithiocarbamate. The inhibitory effect of this complex may arise from interference with the respiration of the fungus by inactivation of the pyruvate dehydrogenase system. The same mode of action was shown primarily in studies on the spoilage fungus, *A.niger* (Lukens, 1971).

Previous studies by Lacey *et al.* (1981) found that Maneb, a fungicide belonging to the dithiocarbamates group, did not delay moulding of hay when applied at 0.5 % doses on hay with a 35 % moisture content. However, Busan 881 had another active ingredient other than the dithiocarbamate, and this may explain its better efficacy on straw.

Adesol 20 was the least effective biocide in controlling fungal populations in bales. The active ingredients in this biocide are ethanol (5 %), dioclyldimethyl ammonium chloride (23 %) and ethane diol (20 %). Previous studies by Lacey *et al.* (1981) found that ethanol and ammonium chloride when used separately did not delay moulding of hay when applied at 0.5 % doses on high moisture content hay. However, they did not investigate effects on straw, or in combinations of treatments as was carried out in this study.

The most effective biocide at controlling fungal activity in situ was Lastil 40. The main active ingredient in this biocide is 2,4,6-Trichlorophenol. This biocide belongs to the phenolic group of biocides. The majority of phenols, especially those containing chlorine are toxic to micro-organisms. They are widely used as industrial biocides for the protection of woods and textiles from fungal attack. The fungicidal action of the various phenols depends on their ability to uncouple oxidative phosphorylation and thus prevent the incorporation of inorganic phosphate into ATP without affecting electron transport. This action probably occurs at the mitochondrial cell wall and enables the cell to continue to respire, but they are soon deprived of the ATP necessary for growth. This is a probable explanation for the mode of action of Lastil 40, although further work would need to be carried out to confirm this. In the Lastil 40-treated straw *E.amstelodami* was the dominant spoilage fungus present. *E.amstelodami* does not pose a health risk and has low cellulose degrading capabilities, so would not adversely affect the structure of the straw. This was one of the reasons why it was chosen as the biocide treatment for the subsequent field trials.

The dominant species isolated from the control samples (no biocide) were *A.fumigatus* and *F.culmorum* in these experiments (NS2). Both of these fungi can cause problems to health. *A.fumigatus* spores are respiratory allergens and can cause the debilitating disease Aspergillosis. *F.culmorum* is a typical field fungus which can produce toxic secondary metabolites (mycotoxins) under conducive environmental conditions. They produce trichothecenes, T2 toxin and fumonisins (Chelkowski, 1989). Langseth *et al.* (1993) found that grain infected with *Fusarium* spp. continued to produce

mycotoxins even though the conditions allowed abundant growth of storage fungi such as *Penicillium* and *Aspergillus* spp. The isolates of *Fusarium* from straw need to be examined for their potential for mycotoxin production. *A.fumigatus* was also abundant. The potential presence of *Fusarium* mycotoxins could be significant if the end use of the straw was for animal consumption.

In the second large scale field trial (NS3), 50 % of the CFUs isolated from the control straw consisted of *A.fumigatus*, after 50 days, while 100 and 150 days of storage all of the fungal populations isolated were *A.fumigatus*. *A.fumigatus* is a thermotolerant species, which has a maximum temperature for growth near 50^0C, and a minimum well below 20^0C, and can therefore survive in a wide range of climates. The temperatures in the top bales fluctuated between 20^0C and 50^0C (see Figure 3.32), ideal conditions for growth of *A.fumigatus*. The other species isolated besides *A.fumigatus* were *F.culmorum* and *A.pullulans*. *F.culmorum* is a mesophilic species with good competitive saprophytic ability (Siran and Chen, 1989). The isolation of this species may have decreased in straw after 50 days storage, due to the marked increase in temperature to approximately 50^0C at about 60 days. *A.pullulans* is a typical primary saprophyte and usually grows actively on the straw at harvest or immediately after harvest. *A.fumigatus* was able to persist because it could tolerate the increases in bale temperatures and is also able to utilise the hemicellulose and cellulose components in straw, perhaps affecting straw quality and structure.

Studies by Chang (1967) and Chang and Hudson (1967) on fungi of wheat straw composts found similar changes in fungal succession and dominance. They found that *A.fumigatus* persisted throughout the storage period regardless of the

temperature fluctuations. They also found that in composts at day zero the temperature was 15^0C, but that was elevated to 68^0C by day 5. A trend was also observed in the first 10 days in all of the straw field trials. An explanation for this increase may also be due to the colonisation by thermophilic species. Chang and Hudson (1967) isolated thermophilic species from composting straw in the first few days, which may validate this explanation. However, thermophilic species were not isolated after 50 days, possibly due to the subsequent decrease in temperature and colonisation by other more competitive species.

In the final large scale field trial (NS4) generally, the wads of straw in nets, chopped straw in nets and biocide sprayed onto straw in nets were all effective treatments for controlling microbial growth when compared to control samples (see Figure 3.35). A possible reason for this was that the additional layer of straw provided a barrier from rainfall, thus maintaining a uniform moisture content across the surface of the bales. The treatment which involved spraying biocide into wads of straw in nets on the top surfaces of dry top bales was also found to have some effect, with a lower maximum temperature being reached than the controls, and a mean moisture content of 24.3 %. The most successful treatment involved the wads of straw on the top of bales where both the number of days at elevated temperature, and increases in moisture content were minimal. However, some ingress of water occurred, responsible for increasing the relative moisture content. Fungal respiration was the major contributory factor for the elevated temperature in the bales.

The Nutri-shield® treatment was reported by the manufacturers to produce a crust over the top surface of the treated bales which would be a very effective water

repellent layer. Visual inspections of the bales during storage, and the temperature data, suggested that this was not happening, with all of the significant periods of rain resulting in major increases in the temperature of these bales. Extensive water ingress occurred, for the dry treatment (15.5 % at baling), the mean moisture content increased to 38.3 % by the end of the storage period. However, this increase in moisture content was not seen at the higher moisture content, and the increase in temperatures in these bales indicates that some drying may have occurred. There were a large number of days at elevated temperature and high moisture content values for the Nutri-shield® treated bales demonstrating the lack of effect of this treatment. At 15 % moisture content Nutri-shield® treated straw had the largest mean total fungal populations when compared to the other treatments (see Figure 3.35) and 50 % of these isolates were again *A.fumigatus*. Nutri-shield® appears to promote the growth of *A.fumigatus*. This may be due to the soya extract component in Nutri-shield® which *A.fumigatus* may be able to utilise. Other species may be able to utilise the soya extract but, due to the competitive nature and thermotolerance, *A.fumigatus* may be able to dominate the stored straw ecosystem. This trend was not apparent for straw baled at 23 % moisture content. A possible explanation could be due to the drying out of the bale, demonstrated by the changes in moisture content (results not shown).

The changes in the structure of the straw samples during storage complemented the mycoflora data, the temperature variations and water ingress observations. The combined data enabled the interaction of each component to be combined to give a clear picture of how and why degradation of straw was occurring.

4.2.4 Effects on straw structure

Generally, there are two types of infra-red spectrometer available for analysis: dispersive and Fourier-Transform (FT) spectroscopy. Each of the machines provides an infra-red spectrum, but by a different method. Dispersive spectroscopy is the commonly used technique. It uses a grating monochromator to disperse the infra-red radiation into its individual frequencies and sweeps from one end of its frequency range to the other. An FT spectrometer, however, irradiates the sample with the whole range of frequencies simultaneously. These interact to form an interference pattern which is subsequently analysed using the mathematical technique called Fourier-Transform spectroscopy. This changes, or transforms, the interference pattern into individual frequencies and intensities.

Dispersive spectrometers suffer from several disadvantages with regard to speed, sensitivity and wavelength accuracy. Since the spectrum is collected by sweeping across the frequency range, and can take several minutes to accumulate, it is not suitable for studying fast reactions or monitoring chromatography column eluates. Sensitivity is poor since most of the infra-red radiation is lost due to the narrowness of the focusing slits instead of passing through the sample. The gratings used to disperse the infra-red radiation are very susceptible to mechanical movement with the result that the accuracy of the incident radiation wavelength can vary appreciably.

The use of FT instruments overcomes all of these difficulties. Since all frequencies are irradiated at once, without the need for dispersion, both the speed and wavelength accuracy are increased. The FT instrument does not focus the

radiation, rather it employs a beam-splitter and mirror system, the working principle of which will not be discussed here. This allows almost all radiation to be collected by the detector, improving the sensitivity dramatically. The interferogram collected by the detector is stored in a digitised form, therefore many spectra can be collated, often within fractions of a second. These can then be averaged to give a much reduced signal to noise ratio.

The traditional methods of obtaining infrared spectra of solid samples are to either use milling agents or compress the sample in an alkali halide disc (Turner and Herres, 1988). Alkali halides are commonly used as supports and diluents in infrared spectroscopy due to their transparency to the infrared radiation. The former method involves grinding a small amount of sample in a mortar and pestle then mixing it with either Nujol (liquid paraffin) or fluorolube (a fluorinated chemical) depending on the spectral region of interest. This suspension is normally smeared between two alkali halide discs and the spectrum collected. The latter method requires the sample under analysis to be ground very finely with approximately a hundred-fold excess of high quality alkali halide (normally KBr). This mixture is then pressed into a disc using a 10 tonne press. These methods, although suitable for fibre extracts, are, in most cases, unsuitable for the fibres themselves. The process of grinding causes changes in the structure of the fibre, such as fragmentation of the polysaccharide chains (Nevell, 1985) and chemical alterations to lignin (Lee and Sumimoto, 1990). Cutting the fibres into small pieces then incorporating these into alkali halide discs is not feasible. The fibres cause fracture lines to occur in the disc making them extremely fragile and brittle. A further problem encountered when analysing such large particles is that a large

proportion of the incident radiation is lost due to scattering which results in a sloping baseline and a generally poor spectrum.

These disadvantages can be overcome by using Diffuse Reflectance Infrared Fourier-Transform (DRIFT) Spectroscopy. This technique has been widely used for the analysis of solids in the UV and visible spectroscopic regions (Kortüm, 1969). Recent advances with FT technology and improved IR detectors, however, have permitted its application in the infrared spectroscopic region. In the field of fibre/cellulose science, the use of infrared spectroscopy, and in particular FT-IR, has seen a large increase over the last decade (Faix, 1986; Berben *et al.*, 1987 and Michell, 1988). More recently DRIFT spectroscopy has been the favoured method (Owen and Thomas, 1989; Anderson *et al.*, 1990; Michell, 1991)

In this study the methodology of Diffuse Reflectance Infrared Fourier-Transform (DRIFT) Spectroscopy was used to analyse the straw samples for NCP and lignin composition in addition to chemical methods. This method enabled the changes in molecular structure of the cellulosic components to be analysed qualitatively and to some extent, semi-quantitatively.

With most perennial or annual, non-wood plant samples, it is necessary to use suitable extraction procedures to remove residual cell contents, particularly soluble phenolics and tannins. Although it was known that cereal straw and treated straw samples would contain low concentrations of residual cell components, previous experience at SCRI with wheat had shown that the concentrations present did not cause any significant effect on the lignin results. Hence, the lignin contents were directly determined on the milled samples.

In order of availability, any carbohydrate in the residual cell contents will be degraded first, followed by degradation of the carbohydrate components of the cell wall or fibre. Degradation of the NCPs usually begins before degradation of cellulose starts, but degradation of both will then progress in parallel. Degradation of the NCPs is not complete before degradation of cellulose begins. As a consequence only the losses of NCP and not cellulose were determined.

The DRIFT spectra of the control and stored bales showed that storage was accompanied by loss of NCP which was evident from the reduced absorbance at 1068 cm^{-1}. The ratio of the lignin-related absorbances at 1595:1510 cm^{-1} changed from <1 in the spectra of the control bales to >1 in the spectra of the stored bales. This suggests that cinnamic acids and non-core, less condensed, lignin has been removed, probably by fungal degradation. Although apparent lignin changes occurred (see Table 3.16) these changes were due to losses of carbohydrate components.

Analysis of the samples from the NS3 trial, by DRIFT spectroscopy show that structural, as well as, compositional changes had taken place (see Figure 3.30). The fingerprint region over the range 1200-900 cm^{-1}, which is dominated by the absorbances due to cellulose and NCPs, exhibited different line shapes and maxima. These are the changes which occur when the secondary and tertiary structure of the straw wall is being disrupted/degraded. There is a reduction in intensity centred at 1720 cm^{-1}, the region of ester carbonyl absorbance, particularly in the spectra of the edge samples. This absorbance is most intense in the spectrum of the samples with moisture contents of 12 % and at a given moisture content, greatest in the core sample. The indication is that ester

hydrolysis is occurring, particularly in the outer and most moist bales. It is known that, during fungal biodegradation of lignocellulosic materials, the micro-organisms secrete esterases, i.e. ester hydrolysing enzymes. Esterases are required if cereal straw is to be degraded by the micro-organism, since the constitutive NCPs, in particular xylan, contain acetyl esters. Since the presence of acetyl groups on xylan and NCPs impedes digestion by secreted xylanases (and hemicellulases), it is necessary for the esterases to be secreted in the initial stages of digestion to facilitate biodegradation.

Analysis of samples from the final field trial (NS4) showed samples undergoing no or low levels of degradation, with very little difference in their DRIFT spectra while those undergoing significant degradation, as determined by the increase in apparent lignin content, also gave increased absorbances at 1595 and 1510 cm^{-1}, the main absorbances associated with lignin.

Unfortunately, pulping of straw from laboratory and field experiments could not be carried out by the associated partners within the overall project.

CHAPTER 5

CONCLUSIONS

5.1 CONCLUSIONS

- The same fungal species were isolated from cv. Ribband and Beaver, but the populations and dominance of individual species varied between the two cultivars.

- As temperature was increased from 10^0C to 30^0C, the number of different species increased and their dominance changed. Colonisation patterns were thus significantly influenced by temperatures.

- Isolation of species also varied with changes in water availability and pH, indicating that interactions between environmental factors markedly influenced rate of deterioration.

- Respiratory activity of the natural mycoflora on straw were significantly influenced by temperature and a_w, with maximum at 30^0C and 0.98 a_w.

- Generally, the respiratory activity of individual species in pure culture on straw had significantly different respiratory rates. *F.culmorum* had the greatest temporal respiratory activity, and *C.cladosporioides* having the lowest activity regardless of a_w.

- Individual species increased their respiratory activity with increasing storage temperature and a_w levels. *F.culmorum* nearly doubled its oxygen consumption at 25^0C when compared to 10^0C storage.

- There was a correlation between temperature x a_w interactions in relation to DML of straw. DML increased with increases in temperature and a_w, both due

to the activity of mixed natural mycoflora on straw, and individual species. Maximum DML were found to be 3.40 % at 30^0C and 0.98 a_w.

- Degradation of carbohydrate occurred during respiration and was confirmed by DRIFT spectral analysis.

- The effect of temperature and a_w levels on degradation of NCP and lignin components of straw were difficult to determine. The greatest increase of apparent lignin was observed at 20^0C. However, the results at 25^0C and 30^0C were less clear. No clear trend could be determined from the data.

- Temperature, a_w and storage period all affected fungal colonisation, dominance and succession on straw. As storage temperature was increased the numbers of different species increased. This trend was observed at all steady-state a_w levels investigated. Total fungal populations increased with the storage time at all temperatures.

- For each of the temperatures as a_w increased DML of straw increased over the storage period. This confirmed that DML was dependent on temperature x a_w interactions.

- All biocides tested reduced fungal isolation when compared to controls. Generally as concentration was increased, the isolation of fungi decreased, suggesting that biocides could be an effective method for controlling fungal growth on stored straw.

- Lastil 40 had the greatest efficacy at inhibiting fungal isolations and growth at all concentrations when compared to controls and other biocides tested.

- All biocides were ineffective at controlling spore germination, but effective at controlling mycelial growth.

- The efficacy of the biocides on fungal respiration was slightly diminished as a_w was increased. They were more effective at 0.95 a_w than 0.98 a_w. Thus efficacy of the biocides were a_w dependent.

- Lastil 40 was the most effective at reducing fungal respiration, decreasing fungal respiration by 66 %, when compared to the controls.

- All biocides reduced DML when compared to controls. Lastil 40-treated straw had the lowest DML at both 0.95 and 0.98 a_w.

- In the preliminary field experiment, all of the biocides significantly decreased the total fungal populations when compared to the controls. However, Lastil 40 treatment was most effective at controlling total fungal populations.

- In the second large field trial (NS3), 50 % of the species isolated from controls were *A.fumigatus*. There was considerable increases in top bale temperatures, which were caused by increased fungal activity due to water ingress. Generally, larger fungal populations were present in the wettest straw bale treatments.

- In the final field trial (NS4) the most effective treatment for the prevention of spoilage was wads of straw on top of the bales. Nutri-shield® treatment

appeared to stimulate microbial growth, with the dominant species being *A.fumigatus*.

- Changes in straw structure complemented the mycoflora data, the temperature variations and water ingress observations. Generally, deterioration of straw corresponded to increases in fungal populations, water ingress and temperature increases.

5.1.2 Future work

- The effect of temperature x a_w interactions on respiration of a wider range of species should be undertaken. A wider range of a_w levels could be tested for each species. The effect of pH on respiration could also be incorporated into such studies.

- Further modification of the respirometer system could enable a larger volume of straw to be tested in each experiment. This would help determine if extrapolations were accurate.

- The storage period for determination of succession in small scale experiments could be extended to one year, with higher temperatures investigated. This would enable the development of thermophilic species and the changes in straw structure could be analysed.

- Further large scale field trials would be needed over longer storage periods and the effect of cultivar could be evaluated in conjunction with the most effective treatments over a wider range of moisture contents. This would enable

information to be gathered on storage quality and rates of deterioration over 12 month storage periods.

APPENDIX

Field experiment (NS3)

Raw data for lignin and non cellulosic polysaccharide content of straw.

No biocide							
Moisture	**Bale**	**Layer**	**Storage**	**Lignin**	**NCP**	**Lignin**	**NCP**
(%)			**(days)**	**(core)**	**(core)**	**(edge)**	**(edge)**
12	1	b	50	14.0 (0.3)	28.6 (0.5)	11.8 (1.2)	34.1 (1.9)
	2	m	50	16.7 (0.4)	38.0 (0.5)	13.9 (0.6)	36.6 (1.8)
	3	t	50	16.1 (0.6)	32.9 (0.5)	13.7 (0.6)	32.4 (2.6)
	4	b	100	14.4 (0.8)	40.0 (1.3)	15.6 (0.2)	28.4 (0.8)
	5	m	100	15.5 (0.7)	37.7 (0.3)	16.8 (0.5)	40.2 (2.9)
	6	t	100	16.2 (0.8)	40.4 (1.3)	18.2 (0.2)	33.5 (2.9)
	7	b	150	13.7 (0.7)	17.6 (2.9)	14.1 (0.8)	13.7 (1.8)
	8	m	150	20.1 (4.2)	25.2 (3.3)	18.4 (3.0)	31.5 (3.3)
	9	t	150	17.9 (5.4)	20.8 (4.9)	25.8 (3.3)	22.7 (2.7)
16	21	b	50	15.0 (0.5)	37.3 (1.3)	13.8 (0.7)	36.5 (1.1)
	22	m	50	15.7 (0.8)	36.7 (0.3)	16.2 (0.7)	37.9 (1.3)
	23	t	50	14.9 (1.2)	36.7 (1.8)	18.3 (0.7)	34.6 (0.2)
	24	b	100	14.0 (0.1)	37.6 (2.2)	14.4 (0.8)	34.7 (1.1)
	25	m	100	15.7 (0.2)	33.9 (0.6)	16.9 (0.6)	33.6 (0.3)
	26	t	100	16.7 (0.4)	35.3 (1.9)	18.9 (0.2)	30.2 (0.7)
	27	b	150	24.1 (3.4)	31.8 (2.0)	22.3 (2.7)	23.9 (1.0)
	28	m	150	20.8 (2.4)	21.0 (5.0)	22.7 (4.2)	25.8 (2.8)
	29	t	150	13.8 (0.5)	15.6 (2.5)	20.2 (0.9)	17.7 (1.6)
20	41	b	50	15.1 (0.5)	33.8 (3.6)	13.7 (1.0)	34.3 (6.5)
	42	m	50	14.6 (0.1)	37.4 (5.4)	13.5 (0.9)	41.3 (2.7)
	43	t	50	16.6 (0.7)	33.9 (2.9)	16.7 (0.4)	33.0 (4.1)
	44	b	100	16.5 (0.5)	33.0 (1.4)	17.3 (0.5)	34.0 (2.6)
	45	m	100	14.8 (1.4)	35.2 (1.2)	16.9 (0.3)	28.3 (3.6)
	46	t	100	15.6 (1.0)	38.9 (1.2)	17.2 (0.4)	26.1 (2.8)
	47	b	150	14.7 (0.8)	18.5 (5.2)	12.8 (0.2)	22.9 (2.5)
	48	m	150	18.6 (5.4)	25.9 (1.2)	13.6 (0.6)	28.0 (3.5)
	49	t	150	14.1 (0.3)	24.4 (0.5)	17.7 (2.5)	21.1 (1.8)
24	61	b	50	16.7 (1.3)	31.0 (2.3)	14.2 (1.0)	32.3 (0.9)
	62	m	50	16.1 (0.5)	38.1 (4.4)	15.7 (0.5)	36.5 (0.7)
	63	t	50	18.3 (0.9)	40.4 (1.6)	18.6 (1.2)	27.9 (3.4)
	64	b	100	16.2 (0.5)	31.4 (0.8)	16.9 (0.7)	32.8 (3.0)
	65	m	100	16.6 (0.05	36.3 (1.7)	16.6 (0.4)	32.6 (1.0)
	66	t	100	17.2 (0.2)	33.8 (3.0)	18.8 (0.4)	38.0 (0.5)
	67	b	150	24.7 (2.2)	17.2 (1.6)	20.5 (1.6)	25.7 (2.1)
	68	m	150	25.1 (1.0)	22.4 (5.7)	22.5 (1.8)	16.6 (8.7)
	69	t	150	22.2 (2.0)	19.8 (3.5)	26.8 (1.7)	19.8 (1.5)

	Biocide						
Moisture	**Bale**	**Layer**	**Storage**	**Lignin**	**NCP**	**Lignin**	**NCP**
(%)			**(days)**	**(core)**	**(core)**	**(edge)**	**(edge)**
12	11	b	50	18.3 (0.3)	35.3 (2.9)	13.5 (1.2)	33.8 (1.1)
	12	m	50	16.9 (0.9)	40.5 (2.4)	14.3 (1.1)	42.4 (1.9)
	13	t	50	16.9 (0.9)	40.5 (2.4)	16.1 (0.9)	34.1 (1.1)
	14	b	100	14.1 (0.6)	44.9 (1.4)	15.9 (0.9)	34.6 (5.3)
	15	m	100	16.4 (0.2)	39.7 (1.5)	12.9 (0.5)	31.9 (2.3)
	16	t	100	15.2 (0.8)	39.0 (2.6)	15.7 (0.3)	35.6 (3.9)
	17	b	150	16.0 (2.4)	12.7 (2.3)	15.3 (0.5	21.6 (4.8)
	18	m	150	15.7 (1.0)	17.3 (4.2)	18.4 (1.2)	13.9 (0.1)
	19	t	150	20.5 (0.7)	21.0 (2.8)	24.5 (3.7)	14.5 (3.8)
16	31	b	50	12.8 (0.5)	31.9 (1.3)	11.2 (1.2)	28.7 (0.1)
	32	m	50	15.2 (0.4)	30.6 (3.6)	14.8 (1.0)	31.6 (2.2)
	33	t	50	15.3 (1.0)	32.9 (1.8)	13.8 (0.8)	37.7 (2.9)
	34	b	100	14.3 (0.3)	29.9 (1.1)	14.9 (0.5)	31.2 (0.7)
	35	m	100	14.6 (1.1)	34.0 (1.5)	14.8 (0.9)	37.8 (2.0)
	36	t	100	15.9 (0.2)	36.9 (1.8)	17.9 (0.5)	35.3 (2.7)
	37	b	150	15.0 (1.0)	14.3 (1.7)	13.3 (2.0)	23.6 (1.3)
	38	m	150	16.4 (3.4)	18.0 (4.4)	17.7 (2.0)	15.6 (1.8)
	39	t	150	25.1 (2.9)	18.4 (5.2)	21.9 (4.3)	10.1 (1.8)
20	51	b	50	14.9 (0.8)	33.8 (0.2)	12.5 (0.8)	31.4 (1.4)
	52	m	50	13.4 (0.9)	32.8 (0.5)	12.4 (0.2)	28.3 (2.9)
	53	t	50	13.4 (0.7)	32.9 (0.5)	15.9 (0.6)	29.4 (3.4)
	54	b	100	14.2 (0.2)	35.6 (3.6)	14.9 (0.4)	32.6 (1.0)
	55	m	100	15.1 (0.8)	34.8 (0.3)	15.8 (1.0)	32.5 (4.0)
	56	t	100	14.1 (0.6)	39.4 (0.8)	16.2 (0.6)	37.8 (1.6)
	57	b	150	17.6 (1.0)	22.8 (2.3)	19.8 (1.6)	16.9 (4.8)
	58	m	150	19.7 (3.0)	20.6 (3.5)	19.5 (0.3)	25.8 (3.8)
	59	t	150	23.7 (0.7)	22.8 (3.9)	25.1 (3.0)	22.5 (2.7)
24	71	b	50	16.0 (0.4)	28.1 (4.0)	13.0 (0.8)	35.1 (0.9)
	72	m	50	15.7 (0.6)	34.1 (4.0)	14.9 (0.7)	28.9 (2.5)
	73	t	50	16.5 (1.0)	34.7 (1.4)	14.2 (0.3)	33.3 (1.2)
	74	b	100	16.4 (0.6)	30.2 (0.2)	15.1 (0.3)	32.2 (3.7)
	75	m	100	13.8 (0.0)	34.7 (0.2)	14.9 (0.4)	31.7 (7.1)
	76	t	100	16.6 (0.3)	38.0 (0.4)	19.5 (1.4)	29.1 (5.3)
	77	b	150	22.5 (2.3)	21.7 (2.5)	21.4 (2.9)	26.0 (1.9)
	78	m	150	18.5 (0.9)	20.8 (3.3)	25.4 (2.3)	20.6 (3.6)
	79	t	150	19.3 (2.0)	24.7 (3.0)	25.2 (2.4)	19.6 (0.3)

The modified respirometer system.

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