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Wheat straw for biofuel production

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1. Abstract

Current commercial cultivars of wheat have been selected according to grain yield and quality, not straw yield or suitability for bioethanol production. There is a lack of data on the relative straw yield of different cultivars of wheat and whether there is variation in digestibility of straw for bioethanol production. It is not known whether the digestibility of wheat straw varies with cultivars, or if this is linked to lodging susceptibility (straw strength). Hence, the relationship between straw digestibility for bioethanol production and lodging resistance (straw strength) was investigated to identify traits with these important parameters with the effect of 'with' and 'without' plant growth regulators (PGRs) and to determine which could be used to select for more efficient bioethanol production. There were no significant differences between cultivars in total biomass production at harvest. However, there were differences in grain yield, straw yield, harvest index, straw glucose yield and straw digestibility. PGR application had no significant effect on total biomass or grain and straw yield; neither did PGRs affect straw glucose yield or straw digestibility but as expected, PGR application significantly reduced cultivar height. There was a negative relationship between cultivar height and straw digestibility which is hypothesised to be due to the greater stem:leaf ratio of taller cultivars. There was no relationship between straw digestibility vs. stem material strength and stem failure wind speed. Although straw digestibility had no relationship with stem failure wind speed, the actual glucose recovered and available for bioethanol production was positively related to stem failure wind speed which is a good indicator for growing dual purpose wheat crop (food and fuel). Moreover, potential bioethanol yield varied between cultivars in both years.

2. Introduction

Production of first generation liquid biofuels from starchy grains (such as wheat and maize) has been controversial due to the potential competition for land for growing food vs. fuel (IEA, 2009; FAO, 2010 and Erdei *et al.*, 2010). Liquid biofuel production could displace food production in the use of land and could also affect the use of natural and agricultural resources (FAO, 2010). Many of the crops currently used as biofuel feedstock require high quality agricultural land and significant inputs of fertilisers, pesticides and water (UN, 2007; Demirbas *et al.*, 2008). This has led to interest in second generation biofuels produced from lignocellulosic materials (*e.g.* *Miscanthus*, cereal straw, and other agricultural by-products) because of its low cost, availability and cellulosic content (Gnansounou *et al.*, 2010; Sims *et al.*, 2010). The second generation biofuel feedstock are rich in cellulose, but that cellulose is very well protected by hemicellulose and lignin and is not easily available for enzymatic digestion (Saha *et al.*, 2005; Kumar and Stroeve, 2009).

In the UK, approximately 12.2 million tonnes of straw was produced from cereals and oilseeds in 2011 (Stoddart and Watts, AHDB 2012). Average harvestable straw yields for wheat, barley and oilseed rape in the UK are estimated to be 2.53 t ha⁻¹, 2.26 t ha⁻¹ and 1.65 t ha⁻¹, respectively (Wilson *et al.*, 2013). Wheat straw makes up 54% of this value and approximately 50% of wheat straw is chopped and incorporated into the soil, rather than being used for animal feed/bedding. Hence, there could be up to 3 million tonnes of wheat straw per annum available for bioenergy production in the UK alone. However, it is important to recognise that, when incorporated into the soil, straw has a nutrient value and also contributes to organic matter content and therefore soil quality (Punter and Woods, 2004; IEEP, 2012). Copeland and Turley (2008) estimated that the nutrient value of wheat straw was £17 per tonne of straw removed, which would rise with increasing fertiliser costs. The long term impacts of removing straw each year on soil quality have yet to be quantified.

Current commercial cultivars of wheat have been selected according to grain yield and quality, not straw yield or suitability for bioethanol production. There is a lack of data on the relative straw yield

of different cultivars of wheat and whether there is variation in digestibility of straw for bioethanol production and whether more digestible straw weaker, and therefore more prone to lodging?

The use of wheat straw for second generation bioethanol production will depend on a number of issues like straw availability, straw quality (lodging) and the economic feasibility of straw digestibility. The recalcitrant nature of lignocellulose is still the bottleneck of modern conversion processes (Himmel *et al.*, 2007). Numerous factors contribute to the recalcitrance of wheat straw during pre-treatment and enzymatic saccharification. One of these factors is the histological variation between different plant tissues and organs. The wheat straw epidermis is thin, but has dense and thick-walled cells with an outer wall coated with a waxy film of cutin-cuticle. The vascular system has xylem tissue with dense lignified structures in the secondary wall, surrounded by a strong sheath of sclerenchyma cells, which have elongated thick lignified cell walls resistant to microbial degradation (Hansen *et al.*, 2011).

Digestibility is not only an important characteristic of cereal straw for second generation biofuel production but also for animal feed. Early studies considering the digestibility of wheat straw were conducted for its use as an animal feed (Capper, 1989; Tolera *et al.*, 2008). Studies revealed differences in straw digestibility between bread wheat cultivars (Knapp *et al.*, 1983; Kernan *et al.*, 1984; Habib *et al.*, 1995). Habib *et al.* (1995) found a highly significant difference in straw digestibility of cultivars, with *in vitro* dry matter digestibility (IVDMD) ranging from 36.40% to 48.36%. Wagner Jensen *et al.* (2011) found significant differences in straw digestibility of 109 currently grown high yielding wheat cultivars. The cultivars exhibited much variation in straw degradability ranging from 258 g kg⁻¹ to 407 g kg⁻¹ of dry matter.

A large number of characteristics determine digestibility of lignocellulosic material, such as lignin content and cellulose crystallinity (Chang & Holtzapple, 2000). Different varieties vary in the proportion of their masses made up by the different components, e.g. the amount of stem and leaves, and these differ in their structure and composition and thus have different digestibility and this means there are differences in overall digestibility (Capper, 1988). Forages and crop residues

consist of a heterogeneous population of cell types each of which have degradation characteristics determined by their location within the plant, by their anatomical features and by the chemical composition of their walls (Travis *et al.*, 1996). Detailed investigation of the relationship between the anatomy and digestibility of forages has demonstrated interactions between cell wall thickness, lignification and other anatomical features in determining the digestibility of different cell types (Minson & Wilson 1994; Travis *et al.*, 1996).

Studies conducted by Kernan *et al.* (1984) observed that differences in the *in vitro* digestibility of organic matter in the leaf and stem fractions of wheat straw contributed more to overall digestibility than the actual proportions of leaf to stem in the straw. Ramanzin *et al.* (1986) observed the same relationship for dry matter degradability of barley straw. Leaves had the highest digestibility followed by chaff, nodes and internodes. However, Capper *et al.* (1989) argued that with a larger range of values, the stem to leaf ratio becomes a major contributor to variation in overall digestibility. Plant height was positively related to the content of cell wall components (Capper *et al.*, 1989; Mathison *et al.*, 1999) and negatively related to microbial degradation in the rumen (Colucci *et al.*, 1992; Mathison *et al.*, 1999). Degradation is closely related to the distribution of cell types (parenchyma, epidermis, and sclerenchyma cells) within leaf and stem, and the thickness of the walls of specific cell types (Goto *et al.*, 1991). Recent studies conducted on wheat straw by Zhang *et al.* (2013) found that straw leaf and stem fractions differed in their compositional profiles and were found to behave differently under enzymatic digestion or saccharification. Pure leaf was hydrolysed significantly better than pure stem. In mixtures, higher leaf:stem ratios always gave a better sugar conversion rate after enzyme digestion or saccharification. Increased straw stiffness may be associated with modified anatomical features of the stems and changed chemical characteristics of the cell walls, which may be expected to decrease degradability of the straw (Travis *et al.*, 1996).

Lodging is the permanent displacement of stems from the vertical and affects all cereal species. Lodging in plants is either due to failure of the stem (Neenan and Spencer-Smith, 1975; Thomas, 1982) or the root system (Crook and Ennos, 1993; Easson *et al.*, 1993). Lodging is affected by

many factors including wind, rainfall, soil, and attributes of the plant as affected by variety, sowing date, seed rate, nitrogen supply, PGRs etc. The effect of these factors on lodging has been very difficult to quantify because of the complexity of the lodging process (Berry and Cameron, 2002).

The process of lodging in wheat in particular has been characterised and well modelled along with the plant characters involved in lodging resistance (Baker, Berry and Spink *et al.*, 1998). The principal factors that are important in lodging in wheat are the leverage related parameters and the stem strength characters. Stem leverage force is determined by parameters such as height at the centre of gravity, ear area, and; natural frequency and shoot number. Stem strength characters includes stem diameter, wall width and material strength of the stem, and the root plate spread and depth.

The lodging model by Berry *et al.* (2003) further develops an existing lodging model by Baker *et al.* (1998) to predict the wind speed at which lodging will occur. The lodging model is based on more important parameters such as stem diameter, plant height and soil type. By using accurate values of drag coefficient and damping ratio, the model has been made more precise, making it easier to compare cultivar resistances and for farmers to select the most suitable cultivar with the lowest lodging risk.

This paper considers biomass production, partitioning to grain and straw, and lodging susceptibility of 15 UK winter wheat cultivars (introduced between 1968 and 2010). Lodging susceptibility and straw strength are assessed using a calibrated model of wheat lodging, as described by Berry *et al.* (2003). The relationship between straw digestibility for bioethanol production and straw strength/lodging resistance is examined.

3. Materials and methods

3.1. Management of the field experiment

Two field experiments were carried out during 2009/10 and 2010/11 at the University of Nottingham, Sutton Bonington Campus near Loughborough, UK. On 20 October 2009, winter wheat was sown at 250 seeds m⁻², using a Wintersteiger plot drill on a stony sandy loam soil (Dunington Heath series). The previous crop was winter oats. The experiment was a randomised block design with 14 cultivars of bread wheat grown in four replicate blocks. The plot size was 24 x 1.6 m with two plots per cultivar in each block (one for crop measurements and one for retaining large quantities of straw for further analysis).

On 13 October 2010, winter wheat was sown at 250 seeds m⁻², using a Wintersteiger plot drill, on a stony sandy loam soil (Dunington Heath series). The previous crop was, once again, winter oats. The same 14 cultivars were grown as in the previous experiment with the addition of Glasgow, a high biomass cultivar, all grown with or without plant growth regulators (PGRs). The experiment was arranged as a split plot with PGR on the main plot and cultivar on the sub plots. The plot size was 24 x 1.6 m with four replicate blocks.

Crop protection chemicals were used prophylactically to minimise weeds, pests and diseases in both years. And soil fertility levels were amended to ensure nutrient availability would not be limiting. The 15 UK winter wheat cultivars were selected to provide a wide range of material in terms of date of introduction, height, lodging resistance and nabim quality group (Table 1).

Table 1: Details of 15 winter wheat cultivars in the experiment with its selection criteria.

Cultivars	nabim Group	Resistance to lodging without PGR	Resistance to lodging with PGR	Height without PGR (cm)	Height with PGR (cm)	Year first listed
Hereward	1	8	9	88	-	1991
Xi 19	1	4	6	97	88	2002
Mascot	1	6	8	93	84	2006
Cordiale	2	8	9	82	76	2004
Battalion	2	7	8	88	82	2007
Sterling	2	6.7	8.3	80	-	2010
Riband	3	8	8	89	-	1989
Zebedee	3	6	6	87	84	2007
Invicta	3	7.2	7.5	93	86	2010
Istabraq	4	6	7	96	88	2004
Ambrosia	4	7	8	88	80	2005
Grafton	4	9	9	79	72	2009
Quartz	4	9	9	75	-	2009
Glasgow	4	6	8	85	74	2005
Maris Widgeon*	-	-	-	-	-	1968

Source: AHDB Recommended Lists for cereals and oilseeds 2009, 2010 and 2011

* limited information available on Maris Widgeon due to date of introduction

3.2. Lodging assessments

A full set of lodging associated measurements in 2009/10 and 2010/11 was taken so that the results could be put into the lodging model by Berry *et al.* (2003). The model allows the strength of the stem and root system to be calculated, along with the failure wind speed of each.

Ten plants were carefully removed from each plot on 1 July, 2010 and 6 July, 2011 when the plants were at Zadoks GS 75. The plants were placed in polythene bags and stored at 4°C until laboratory analysis was complete.

In the laboratory, the main shoot was isolated and the principal plant characters involved in stem lodging, as identified by Berry *et al.* (2000) were measured, including natural frequency, number of ears per plant, ear area, height at centre of gravity and length, diameter, wall width and breaking strength of the bottom two internodes (internodes 1 and 2). The detailed methodology for these measurements is described by Berry *et al.* (2000). Calculation of stem failure wind speed, stem material strength and stem leverage were done using calculations described in Berry *et al.* (2000).

3.2.1. Stem Leverage Characters

The height at the centre of gravity and main stem ear area, along with the natural frequency, are used to calculate stem leverage (Equation 3.1). The centre of gravity was determined by balancing the main stem on an extended finger, so that the point of balance could be determined. The height at the centre of gravity was recorded as the distance from the base of the stem to the balance point. Natural frequency was measured on the main stem, which was clamped at the base. The top of the shoot was displaced to a distance of approximately 10 cm from the vertical and then released, and time taken for three oscillations was recorded. Natural frequency (n) is then calculated as the number of oscillations per second. Ear area was measured using LI-COR leaf area meter. Stem leverage (B) is expressed by Equation 3.1.

Equation 3.1: Leverage on internode one (after Baker *et al.*, 1998)

$$B = \frac{1}{2} \rho A C_D V_g^2 X \left(1 + \frac{g}{(2\pi n)^2 X} \right) \left(1 + e^{-\pi \delta \frac{\sin(\pi/4)}{\pi/4}} \right)$$

Where:

ρ = density of air (1.2 kg m⁻³)

A = main stem ear area (m²)

C_D = drag coefficient (taken as 1)

V_g = wind gust speed (taken as 13 m s⁻¹)

X = height at the centre of gravity (m)

g = acceleration due to gravity (9.81 m s⁻²)

n = natural frequency (Hz)

δ = damping ratio (taken as 0.08)

Leverage on internode two (B_2) was also calculated. This is defined by Equation 3.2.

Equation 3.2: Leverage on internode two (after Baker *et al.*, 1998)

$$B_2 = B \left(\frac{X - h_1}{X} \right)$$

Where:

B = leverage on internode one

X = height at the centre of gravity (m)

h_1 = internode one length (m)

The stem failure wind speed (V_{gs}) can be calculated using stem leverage and stem failure moment values described in Equation 3.3.

Equation 3.3: Stem failure wind speed for internode one (after Berry *et al.*, 2000)

$$V_{gs1} = \sqrt{\frac{2B_{S1}}{(\rho AC_D X) \left(1 + \frac{g}{(2\pi n)^2 X}\right) \left(1 + e^{-\pi\delta} \frac{\sin(\pi/4)}{\pi/4}\right)}}$$

Where:

V_{gs1} = stem failure wind speed (m s⁻¹)

B_{S1} = internode one failure moment (Nm)

Internode two failure wind speed (V_{gs2}) is calculated using Equation 3.4.

Equation 3.4: Stem failure wind speed for internode two (after Berry *et al.*, 2000)

$$V_{gs2} = \sqrt{\frac{2B_{S2}}{B\left(\frac{X-h_2}{X}\right) (\rho AC_D X) \left(1 + \frac{g}{(2\pi n)^2 X}\right) \left(1 + e^{-\pi\delta} \frac{\sin(\pi/4)}{\pi/4}\right)}}$$

Where:

B_{S2} = internode two failure moment (Nm)

3.2.2. Stem Strength Characters

The strength at the point of buckling of the internode may be described using the term internode failure moment, calculated from internode length and breaking strength. Internode material strength is calculated from internode failure moment, radius and wall width.

Internode breaking strength was measured by holding the internode against a Y-shaped brace that was clamped to the bench. The two nodes were supported by the metal prongs and a Weighmate digital scale (10 kg x 10 g; 1 kg = 9.81 Newtons) was pulled at an even rate to the point of internode failure. The force recorded just prior to the buckling of the internode was taken as its breaking strength. Internode length was measured from the mid-point of one node to the mid-point of the next node.

Internode failure moment (Equation 3.5) is the term of choice when describing stem strength, as this provides an estimate of internode strength that is independent of the length of the internode.

Equation 3.5: Internode one failure moment (after Baker *et al.*, 1998)

$$B_{S1} = \left(\frac{h_1 F_{S1}}{4} \right)$$

Where:

B_{S1} = internode one failure moment (Nm)

h_1 = internode one length (m)

F_{S1} = internode one breaking strength (N)

Internode radius and wall width are used with the breaking strength to calculate internode material strength. The diameter was measured using digital callipers at the midpoint of each internode then divided by two to give the radius. The internode was cut transversely at the centre using a knife and the wall width was measured using digital callipers; the internode was then rotated 90° and the wall width were measured again. The mean of these two measurements was taken as the internode wall width. This method was employed due to high natural variability in this particular character. Internode material strength (σ) is calculated using Equation 3.6.

Equation 3.6: Internode material strength (after Berry *et al.*, 2000)

$$\sigma = \frac{F_S h a}{\pi(a^4 - (a-t)^4)}$$

Where:

F_S = internode one breaking strength (N)

h = internode length (m)

a = internode radius (m)

t = internode wall width (m)

3.3. Assessment of biomass production and partitioning

When the crops were fully mature, a 0.5 x 0.5 m area was randomly sampled from each plot and cut at soil level with secateurs. The samples were removed to the laboratory for detailed analysis. The total biomass of the sample was determined by drying to constant mass, and then the ears were separated from the stems and threshed so that straw, grain and chaff weight could be

determined. Harvest index was determined as the proportion of total biomass in the grain and straw index as the proportion of total biomass in the straw.

3.4. Laboratory analysis of straw digestibility

Straw digestibility is expressed as the percentage of total available glucose in the residue released during enzyme hydrolysis. Straw digestibility consisted of four steps: milling, pre-treatment, acid hydrolysis and enzyme hydrolysis.

3.4.1. Milling

To obtain homogeneous samples, wheat straw comprising stem and leaf components was knife milled with a 1 mm mesh size (P15 mill, Fritsch GmbH) generating particle sizes of 200–700 microns.

3.4.2. Pre-treatment of milled straw

One gram of milled wheat straw from each of the winter wheat cultivars was added to 10 ml of 1% H₂SO₄ (0.1M) and incubated for 20 minutes at 121°C using an autoclave. Samples were then brought to pH 4.5–4.8 by the addition of distilled H₂O then filtered through Whatman filter paper (110 mm Dia). The filtrate was discarded and the retained residue was dried overnight at 40°C in an oven.

3.4.3. Acid hydrolysis of pre-treated straw residue

Thirty milligrams of pre-treated dry wheat straw residue from each of the selected winter wheat cultivars were placed in a heat resistant screw cap tube and 1 ml of 12M H₂SO₄ was added and then incubated at 37°C for 1 hour. Samples were then diluted to 1M H₂SO₄ by the addition of 11 Eml distilled H₂O, and incubated at 100°C for a further 2 hours. After cooling under tap water, acid hydrolysed samples were then aliquoted and kept at -80°C until further high performance anion exchange chromatography (HPAEC) analysis.

3.4.4. Enzyme hydrolysis of pre-treated straw residue

Two hundred milligrams of cellulase enzyme (EC 3.2.1.4) from *Trichoderma reesei* ATCC 26921 (Sigma-Aldrich) was dissolved in 20 ml of 50 mM NaCitrate buffer (pH 4.8) and dialysed (molecular cut off: 12-14000 Daltons) overnight at 4°C with constant stirring in 2 litres of 50 mM NaCitrate buffer to remove any traces of sugars and other low molecular weight impurities.

Then, 200 mg of pre-treated dry wheat straw residue was added to 36 ml of the 50mM NaCitrate buffer (pH 4.8) in a screw cap falcon tube and 4 ml of the dialysed enzyme solution was added to give an enzyme activity equivalent to 40 FPU/g of biomass, at a concentration of 2 FPU/ml. Samples were then incubated at 50°C on an orbital shaker at 150 rpm for 72 hours. Aliquots of 1 ml were taken at time 0 (as soon as the enzyme was added) and then after 2, 4, 6, 24, 48 and 72 hours of incubation. To stop further enzyme activity, the sample was briefly centrifuged at 1000 rpm for 30 seconds and the supernatant was quickly frozen at -80°C until further HPAEC analysis.

3.4.5. Sugar analysis using High Performance Anion Exchange Chromatography (HPAEC)

Sugars were determined by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (DIONEX ICS - 3000, UK) using a CarboPac PA20 column with a 50 mM NaOH isocratic system and flow rate of 0.5 ml min⁻¹ at 30°C. Glucose, xylose, arabinose and galactose were used as standards with mannitol as internal standard.

A standard curve was plotted for each sugar and the trend line equation was used to calculate the amount of sugar content and fermentable glucose yield in grams per litre for each straw sample. The best fitted line was calculated using Excel software. The linear equation obtained from the standard curve was used to find out the concentration of sample glucose in g L⁻¹. Then further the value was used to calculate mg of glucose per gram of straw.

3.5. Statistical analysis

Analysis of variance (ANOVA) procedures appropriate for the experimental design and regression analyses were carried out using GenStat for Windows, 15th Edition (Lawes Agricultural Trust). All data were converted to a per m² basis prior to statistical analysis.

4. Results

4.1. Biomass production and partitioning

Total biomass production was assessed in both years. There were no significant differences between cultivars in total biomass production in 2010 and 2011 (Tables 2 and 3). However, there were significant differences in grain yield in 2010 ($P=0.047$) and 2011 ($P<0.001$) with Cordiale producing the highest grain yield in 2010, Glasgow producing the highest grain yield in 2011 and Maris Widgeon produced the lowest yield in both years (Tables 2 and 3). There were large differences between cultivars in straw yield in 2010 ($P=0.005$) and 2011 ($P<0.001$) with Maris Widgeon, the only non-semi dwarf, produced the most straw in both years (Tables 2 and 3). Cultivars differed significantly in the quantity of chaff produced in 2010 ($P=0.022$; Table 2) and 2011 ($P=0.002$; Table 3). PGR application had no significant effect on total biomass, grain yield, straw yield or chaff biomass and there were no significant interactions between cultivar and PGR in 2011.

With the exception of Maris Widgeon, all cultivars had a harvest index above 0.53, the highest being 0.57 in 2010 ($P<0.001$) (Cordiale, Grafton Mascot and Quartz; Table 2) and 0.59 in 2011 ($P<0.001$) (Grafton and Riband; Table 3). Considering straw yield as a proportion of total biomass (straw index), as expected, Maris Widgeon had the highest straw index (0.48 in 2010 and 0.46 in 2011) and Cordiale (0.29 in 2010 and 2011) the lowest in both years (Tables 2 and 3) and with other cultivars ranging from 0.30-0.33 ($P<0.001$; Tables 2 and 3). PGR application had no significant effect on harvest index and straw index and there were no significant interactions between cultivar and PGR in 2011.

Table 2: Total biomass production and yield components of 14 winter wheat cultivars in 2010.

Cultivars	Total Biomass (g m ⁻²) (2010)	Grain Yield (g m ⁻²) (2010)	Straw Yield (g m ⁻²) (2010)	Chaff Biomass (g m ⁻²) (2010)	Harvest Index (2010)	Straw Index (2010)
Ambrosia	1564	877	505	154	0.56	0.32
Battalion	1565	872	511	164	0.55	0.32
Cordiale	1846	1061	553	201	0.57	0.29
Grafton	1378	788	422	152	0.57	0.30
Glasgow	-	-	-	-	-	-
Hereward	1540	829	506	176	0.53	0.32
Invicta	1540	816	537	160	0.52	0.35
Istabraq	1492	829	486	152	0.55	0.32
Maris Widgeon	1452	596	709	139	0.41	0.48
Mascot	1492	863	473	152	0.57	0.31
Quartz	1411	813	424	160	0.57	0.30
Riband	1703	964	551	174	0.56	0.32
Sterling	1391	767	441	156	0.54	0.32
Xi 19	1779	987	581	198	0.55	0.32
Zebedee	1407	797	439	149	0.56	0.31
Mean	1540	847	510	163	0.54	0.33
<i>P</i>	0.265	0.047	0.005	0.022	<0.001	<0.001
SED	181.3	110.5	63.2	17.29	0.0155	0.0130
df	39	39	39	39	39	39

Table 3: Total biomass production and yield components of 15 winter wheat cultivars in 2011.

Cultivars	Total Biomass (g m ⁻²) (2011)	Grain Yield (g m ⁻²) (2011)	Straw Yield (g m ⁻²) (2011)	Chaff Biomass (g m ⁻²) (2011)	Harvest Index (2011)	Straw Index (2011)
Ambrosia	1747	1005	559	177	0.57	0.31
Battalion	1585	893	513	172	0.56	0.32
Cordiale	1503	878	446	172	0.58	0.29
Grafton	1573	929	469	169	0.59	0.29
Glasgow	1788	1048	522	207	0.58	0.29
Hereward	1653	880	556	209	0.53	0.33
Invicta	1686	938	556	176	0.55	0.33
Istabraq	1759	977	578	194	0.55	0.32
Maris Widgeon	1589	670	743	165	0.42	0.46
Mascot	1657	923	545	178	0.55	0.32
Quartz	1507	861	459	169	0.57	0.30
Riband	1614	956	494	160	0.59	0.30
Sterling	1434	820	446	157	0.57	0.31
Xi 19	1618	907	520	185	0.55	0.32
Zebedee	1635	946	508	172	0.57	0.31
Mean	1623	909	528	178	0.56	0.32
<i>P</i>	0.23	<0.001	<0.001	0.002	<0.001	<0.001
SED	121.8	68.3	44.08	12.42	0.0078	0.0070
df	56	56	56	56	56	56

4.2. Straw glucose yield and straw digestibility

Straw glucose yield differed significantly between cultivars with Quartz recording the highest glucose yield and Maris Widgeon the least in both years ($P < 0.001$; Table 4). Straw digestibility is expressed as the percentage of total available glucose in the residue released during enzyme hydrolysis. Moreover, straw digestibility also differed significantly between cultivars in 2010 and 2011 ($P < 0.001$; Table 5) with Quartz recording the highest straw digestibility in 2010 (60.32%) and Cordiale the highest in 2011 (67.46%) and with Maris Widgeon recording the lowest digestibility in both years (Table 5). PGR application had no significant effect on straw glucose yield and straw digestibility and there were no significant interactions between cultivar and PGR in 2011.

Table 4: Glucose yield of 6 winter wheat cultivars in 2010 and 7 winter wheat cultivars in 2011.

Cultivars	Glucose Yield (mg gm ⁻¹) (2010)	Glucose Yield (mg gm ⁻¹) (2011)
Cordiale	344	390
Hereward	324	375
Istabraq	-	369
Maris Widgeon	244	315
Quartz	374	404
Riband	336	380
Zebedee	339	380
Mean	327	373
<i>P</i>	<0.001	<0.001
SED	9.71	10.72
df	15	24

Table 5: Straw digestibility of 6 winter wheat cultivars in 2010 and 7 winter wheat cultivars in 2011.

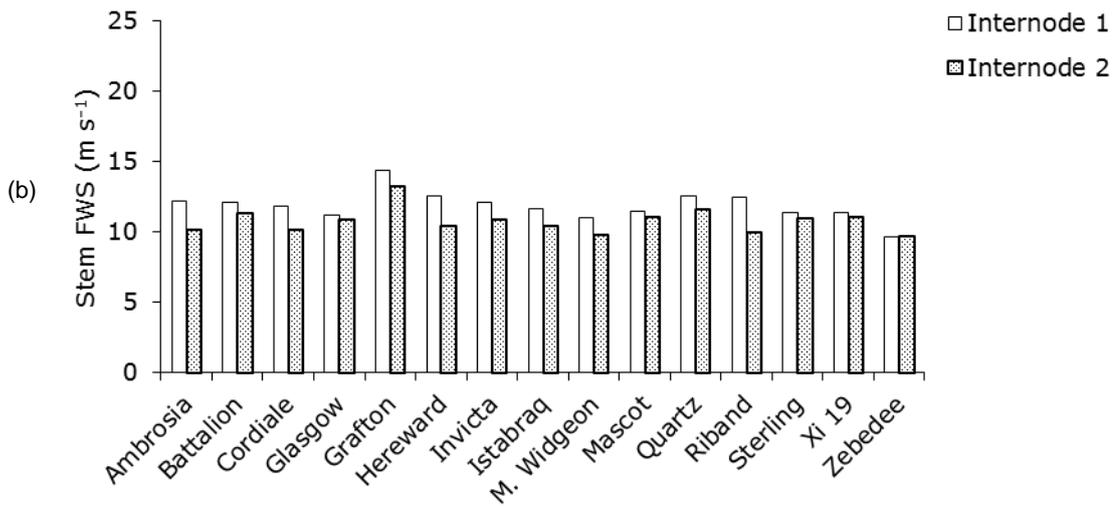
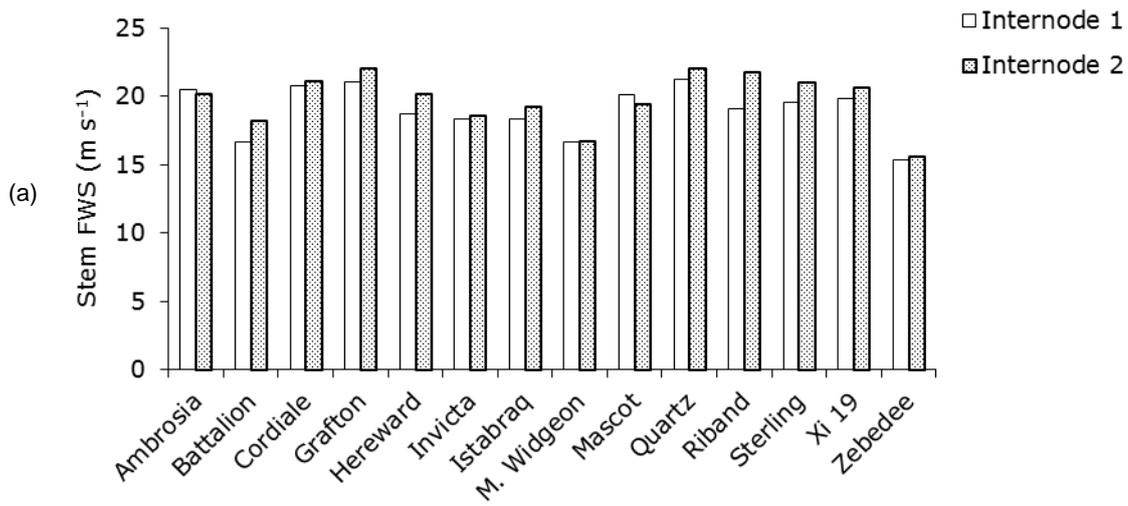
Cultivars	Straw Digestibility (%) (2010)	Straw Digestibility (%) (2011)
Cordiale	54.02	67.46
Hereward	50.63	65.98
Istabraq	-	62.74
Maris Widgeon	38.65	54.13
Quartz	60.32	67.15
Riband	53.08	63.95
Zebedee	53.99	65.95
Mean	51.78	63.91
<i>P</i>	<0.001	<0.001
SED	1.767	2.516
df	15	24

4.3. Lodging assessments

The lodging model (Berry *et al.*, 2000) uses crop measurements to predict the wind speed at which lodging will occur. Cultivars differed significantly for stem failure wind speed for internode 1 and 2 in 2010 ($P<0.001$; Figure 1 (a)) with cultivars ranking similarly for internode 2 in 2011 (Figure 1 (b)). Stem failure wind speed values were lower overall with significant differences for internode 1 in 2011 ($P<0.001$; Figure 1 (b)).

The cultivars most prone to stem lodging were Zebedee, followed by Battalion and Maris Widgeon in 2010 (Figure 1 (a)). The most resistant was Quartz in 2010 (Figure 1 (a)) and Grafton in 2010 and 2011 (Figure 1 (a) and (b)). There was a trend for PGR to increase stem failure wind speed for both internodes, but the differences were not significant and there were no significant interactions between cultivar and PGR for either internode in 2011 (Figure 1 (c) and (d)).

The component of stem failure wind speed most likely to be related to digestibility of the stem is the stem material strength (Figure 2). Differences between cultivars in stem material strength were not significant for internode 1 and 2 in 2010 (Figure 2 (a)). Cultivars ranked similarly in terms of stem material strength for internode 2 (Figure 2 (b)) but significantly differed for internode 1 in 2011 ($P=0.002$; Figure 2 (b)). PGR had an increasing and decreasing effect and had no significant effect on stem material strength for both internodes and there were no significant interactions between cultivar and PGR for either internode (Figure 2 (c) and (d)).



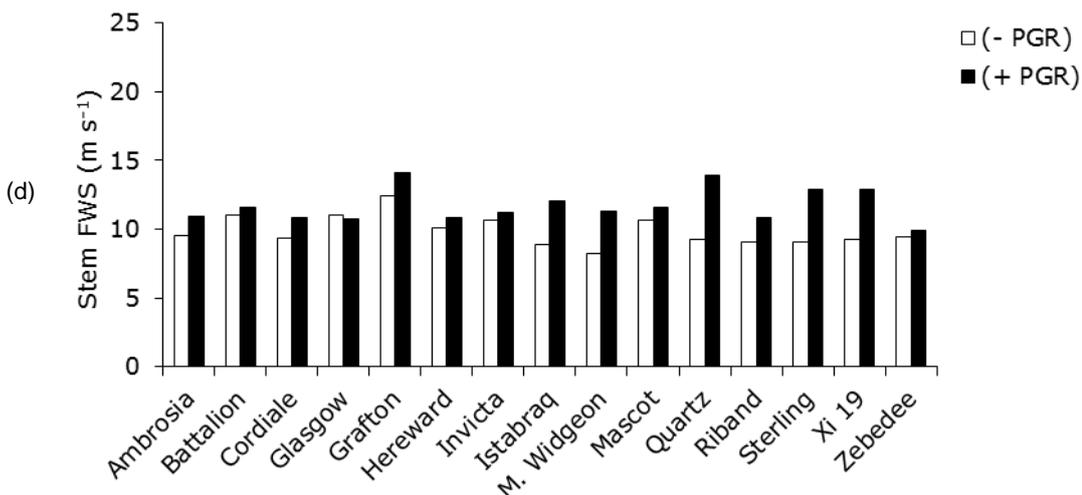
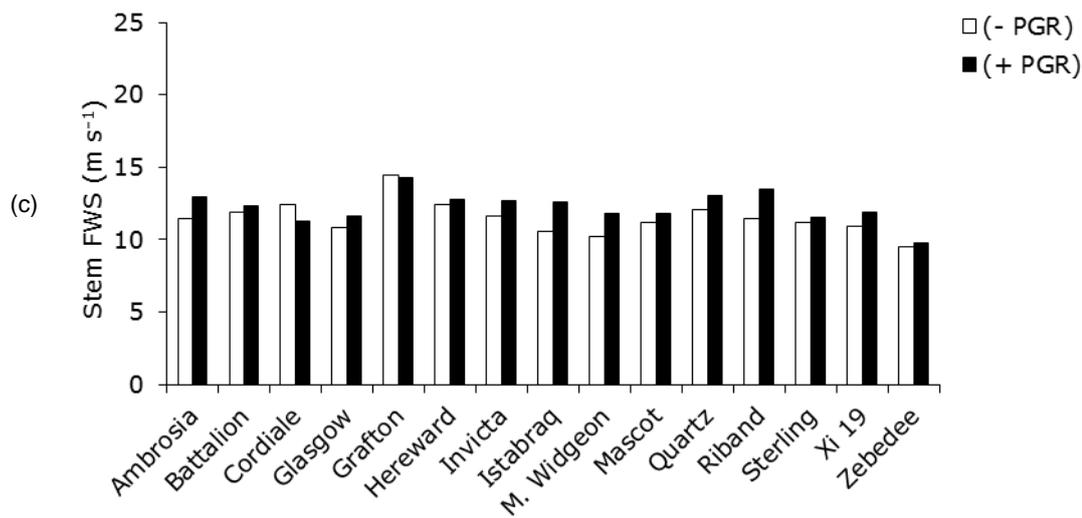
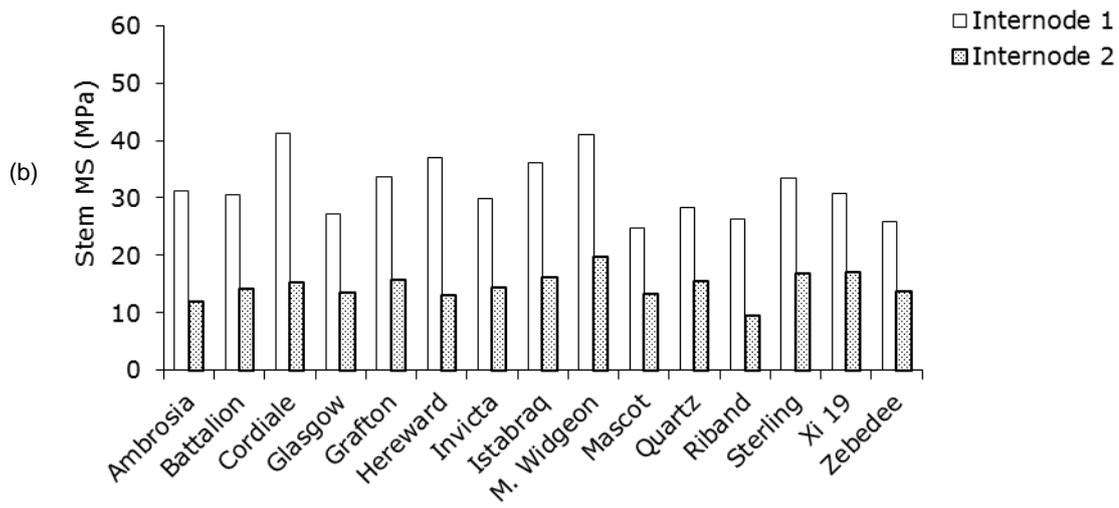
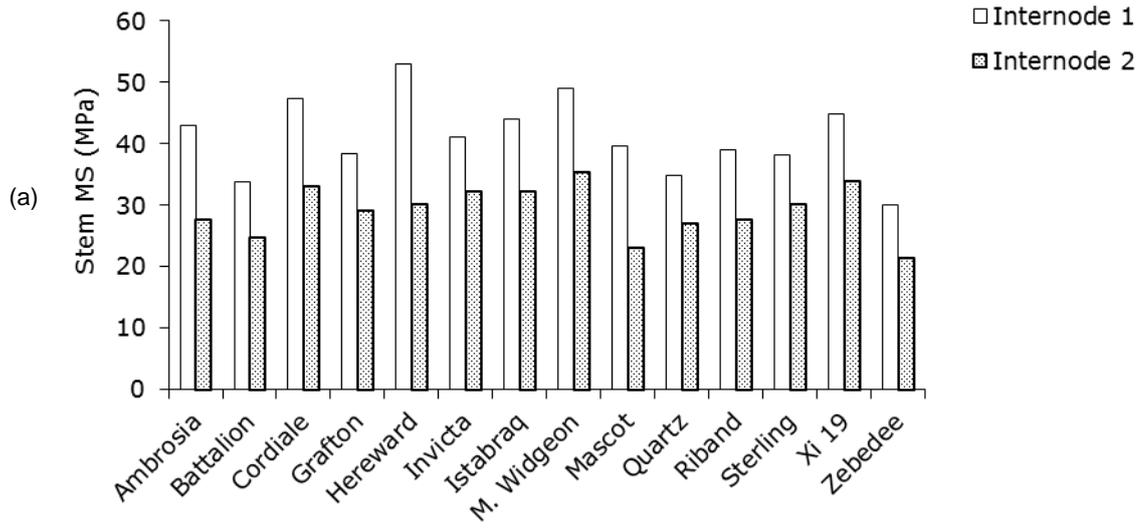


Figure 1: (a) Internode 1 stem failure wind speed (SED=1.214, df=39) and internode 2 stem failure wind speed (SED=1.402, df=39) of 14 winter wheat cultivars in 2010. (b) Effect of cultivar on internode 1 stem failure wind speed (SED=0.752, df=56) and internode 2 stem failure wind speed (SED=0.93, df=56) of 15 winter wheat cultivars in 2011. (c) Effect of cultivar and PGR on internode 1 stem failure wind speed (SED=1.056, df=57.46) of 15 winter wheat cultivars in 2011. (d) Effect of cultivar and PGR on internode 2 stem failure wind speed (SED=1.346, df=49.77) of 15 winter wheat cultivars in 2011.



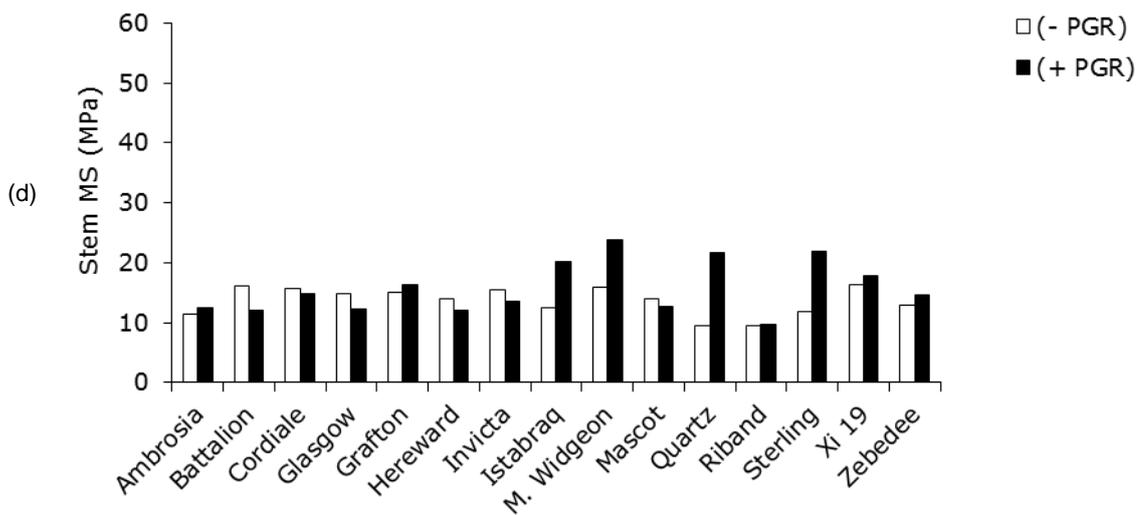
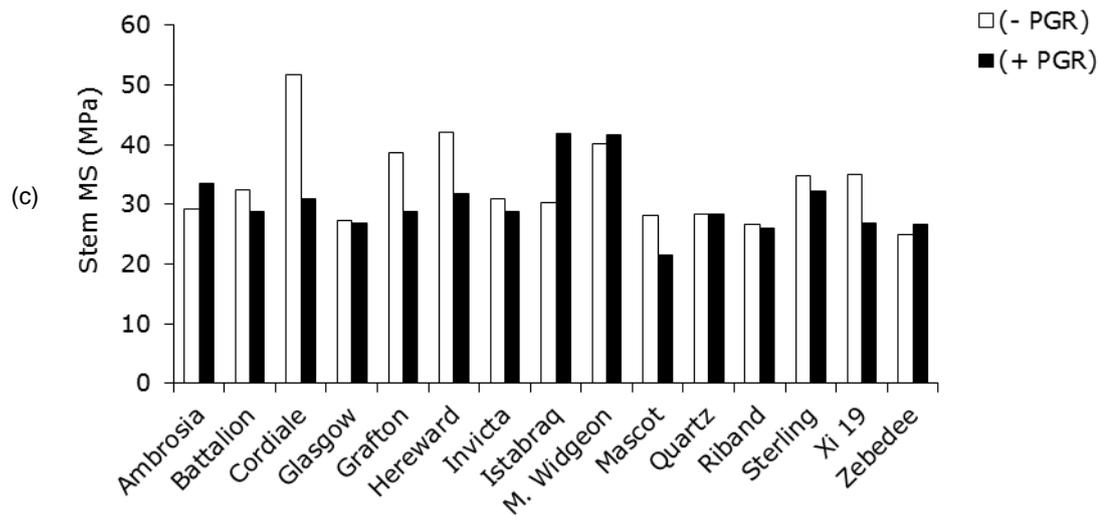


Figure 2: (a) Internode 1 stem material strength (SED=7.83, df=39) and internode 2 stem material strength (SED=4.27, df=39) of 14 winter wheat cultivars in 2010. (b) Effect of cultivar on internode 1 stem material strength (SED=4.33, df=56) and internode 2 stem material strength (SED=3.11, df=56) of 15 winter wheat cultivars in 2011. (c) Effect of cultivar and PGR on internode 1 stem material strength (SED=5.99, df=57.87) of 15 winter wheat cultivars in 2011. (d) Effect of cultivar and PGR on internode 2 stem material strength (SED=4.56, df=44.97) of 15 winter wheat cultivars in 2011.

4.4. Relationship between straw digestibility and lodging resistance

When straw digestibility was regressed against stem material strength, there was no significant relationship for either internode in 2010 or 2011 analysed alone. PGR application had no significant effect on the relationship in 2011. Stem material strength is not the only determinant of lodging susceptibility as the leverage force on the stem also needs to be considered.

A key component of leverage force is cultivar height. When cultivar height was regressed against straw digestibility, a significant negative relationship was detected in both years ($P < 0.001$, $R^2 = 0.7049$; Figure 3 (a)) and ($P < 0.001$, $R^2 = 0.373$; Figure 3 (b)). PGR application had no significant effect on the relationship in 2011 (Figure 3 (b)).

Stem strength and leverage force are used to calculate stem failure wind speed (the wind speed at which the stem is predicted to fail). When straw digestibility was regressed against stem failure wind speed, there was no significant relationship for either internode in 2010 or 2011 analysed alone. PGR application had no significant effect on the relationship in 2011.

When glucose yield was regressed against stem failure wind speed, a significant weak linear relationship was detected for internode 1 ($P = 0.022$, $R^2 = 0.216$; Figure 4 (a)) in 2010 but there were no relationship for internode 2 in 2010 and for either internode in 2011. PGR application had no significant effect on the relationship in 2011.

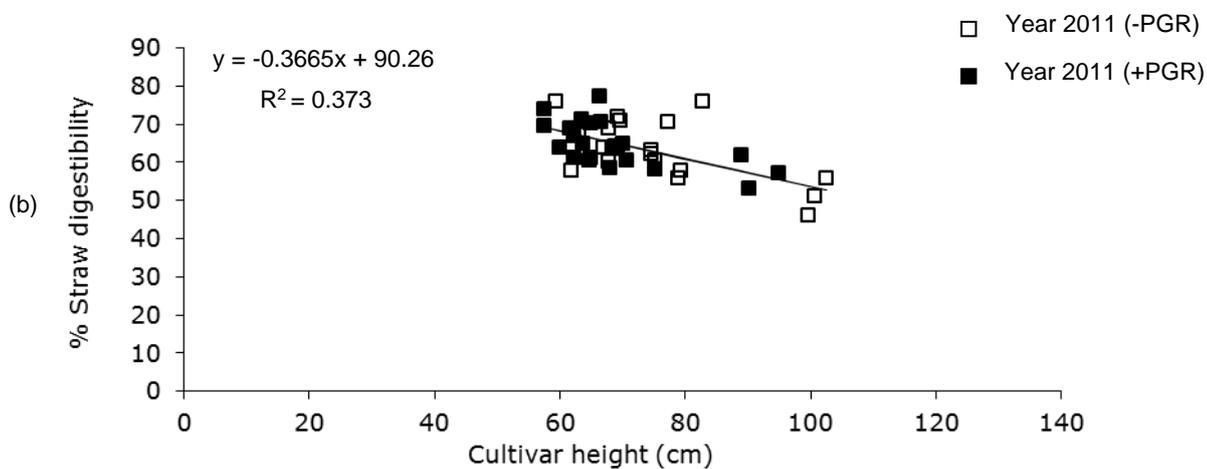
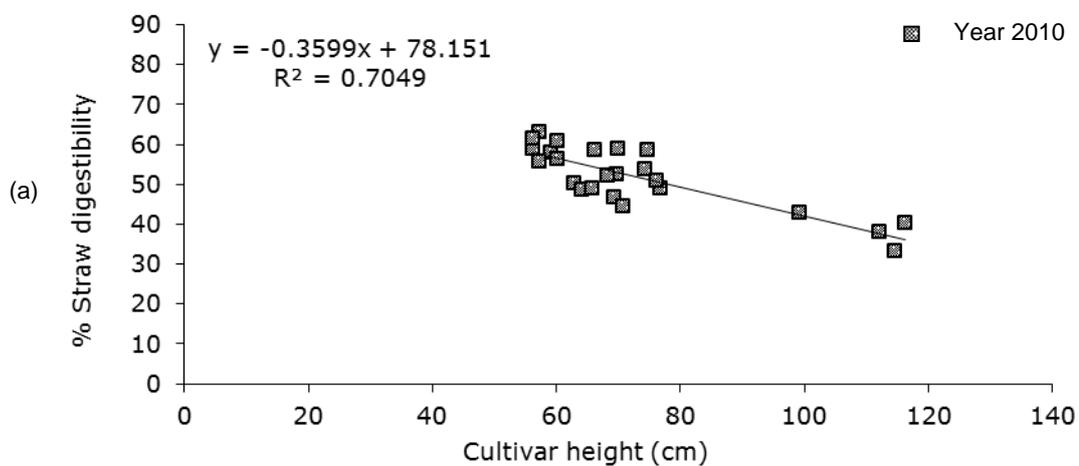


Figure 3: (a) Relationship between cultivar height and % straw digestibility of 6 winter wheat cultivars in 2010. (b) Relationship between cultivar height and % straw digestibility of 7 winter wheat cultivars in 2011.

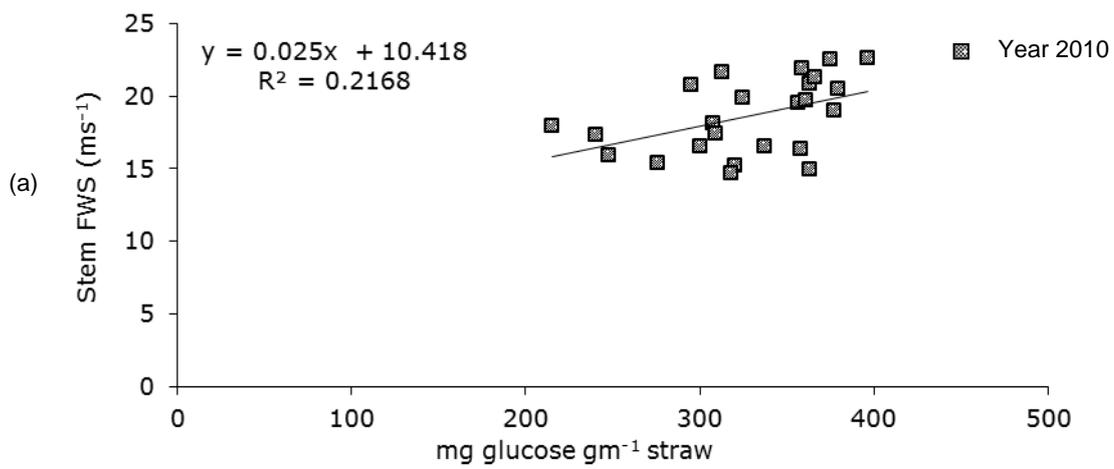


Figure 4: (a) Relationship between glucose yield and internode 1 stem failure wind speed of 6 winter wheat cultivars in 2010.

5. Discussion

The 15 cultivars selected produced similar amounts of biomass per unit area but contrasted for partitioning of that biomass to grain, straw and chaff and therefore had significantly different grain and straw yields. This is not surprising as several reviews of yield progress in wheat have shown that recent improvements in yield have come mainly from increases in harvest index rather than total biomass production (e.g. Shearman *et al.*, 2005). Maris Widgeon, the only non-semi-dwarf cultivar in the experiment, produced similar amounts of biomass as the other cultivars but, as expected, had a smaller harvest index and hence less grain yield and greater straw yield than the other cultivars.

Pre-treatment was selected to achieve 50% subsequent saccharification of glucose from a standard wheat cultivar. This was to identify variations in digestibility between cultivars. Glucose yield from pre-treated straw residue after enzyme hydrolysis varied between cultivars in both years. This is a similar finding to Knapp *et al.* (1983) who reported significant differences between cultivars of winter wheat in the amount of reducing sugars released by enzyme saccharification with *Trichoderma reesei*. In contrast, Larsen *et al.* (2012) found no significant cultivar differences with enzyme saccharification either in terms of glucose, xylose nor total sugar released from straw. With similar level of glucose content from straw residue, Quartz had the highest glucose yield and Maris Widgeon the least from pre-treated straw residue after enzyme hydrolysis in both years. Lindedam *et al.* (2012) reported a similar range of C6 sugar (glucose) release ranging from 0.21-0.22 g g⁻¹ of straw from twenty wheat cultivars. Saha *et al.* (2005) reported a maximum value of 550 mg g⁻¹.

Straw digestibility is expressed as the percentage glucose released by enzymes. Straw digestibility varied between cultivars in both years and PGR application had no effect on straw digestibility in 2011. Average straw digestibility was higher in 2011 (63.91%) than 2010 (51.78%). Jensen *et al.* (2011) identified differences between winter wheat cultivars with respect to degradability of straws from 106 winter wheat cultivars which exhibited differences in degradability ranging from 258 g kg⁻¹ to 407 g kg⁻¹ of dry matter by using *in vitro* enzymatic solubility (EFOS) assay which was been

developed to assess feed value. EFOS assay correlates with other assays such as the recently developed high-throughput pre-treatment and enzymatic saccharification developed by Selig *et al.* (2010). White *et al.* (1981) previously also found significant differences in degradability in a set of 25 winter wheat cultivars.

Quartz consistently had higher glucose yield with the highest straw digestibility with 60.32% followed by Cordiale (54.02%) in 2010. But surprisingly, Cordiale had the highest straw digestibility with 67.46% followed by Quartz (67.15%) in 2011. But moreover, Cordiale also had the highest glucose yield after Quartz in both years. However, Habib *et al.* (1995) also found a highly significant difference in digestibility by using *in vitro* dry matter digestibility (IVDMD) by 48 h *in vitro* fermentation technique developed by Tilley and Terry (1963) ranging from 36.40% to 48.36% in 15 wheat cultivars. Interestingly, Maris Widgeon the only non-semi-dwarf with high straw yield had the least straw digestibility (38.65% and 54.13% in 2010 and 2011, respectively) in both years as well had the least glucose yield in both years. This was in agreement with Capper (1988) who suggested that taller cultivars would have more stem than shorter cultivars and this would theoretically result in lower digestibility. Tolera *et al.* (2008) also reported that there were differences in digestibility caused by cultural practice. Cordiale and Quartz both a semi-dwarf with the greatest grain yield along with greatest straw yield had the highest straw digestibility in both years. Keman *et al.* (1984) and Tolera *et al.* (2008) reported that the digestibility of different parts or components of the plant varies and part of the reason for the difference in digestibility of the different cultivars may be due to different ratios of the parts of the plants (leaves vs. stem). Travis *et al.* (1996) who also reported that increased straw stiffness may be associated with modified anatomical features of the stems and changed chemical characteristics of the cell walls, which may be expected to decrease degradability of the straw.

There was no evidence that straw digestibility was related to lodging susceptibility. When data from 2010 or 2011 were analysed separately, no relationship was found between straw digestibility and stem material strength. However, suggests that cultivars with the highest straw digestibility are not necessarily with more stem material strength and may less likely to get lodged. Baker *et al.*

(1998) reported that stem lodging risk increases significantly if the stem material strength falls below 20 MPa. Travis *et al.* (1996) found that wheat and barley varieties that were more resistant to lodging had higher *in vitro* degradability of the basal internode than susceptible varieties.

Stem material strength is only one component of overall stem strength which is also dependent on factors such as internode length. Stem failure wind speed is the force of wind required to cause the internode to lodge and is hence a better indicator of lodging risk than stem material strength. There was no evidence that straw digestibility was related to stem failure wind speed, *i.e.* lodging susceptibility, for either internode in 2010 or 2011 when analysed alone. Berry *et al.* (2000) reported that stem lodging risk increases significantly if the stem failure wind speed falls below 20–25 m s⁻¹ and the wheat must withstand wind gusts of up to 40 m s⁻¹.

Although, straw digestibility had no relationship between stem failure wind speed but the actual glucose recovered and available for bioethanol production was positively related to stem failure wind speed for internode 1 in 2010. Moreover, PGR had no significant effect on the relationship in 2011.

A key component of leverage force is cultivar height. When regressed against straw digestibility, a negative relationship was detected in both years. This agrees with Jensen *et al.* (2011) who reported that degradability of straw decreased with height as more degradable leaves constitute a larger part of the straw in shorter cultivars. Thus, this indicates that taller wheat cultivars are less digestible. Lindedam *et al.* (2012) suggested that cultivar-specific relationships of leaf and stem sugar yield is more important in predicting the overall sugar yield than the leaf-to-stem ratio. Taller plants had a preferred structure for pre-treatment and enzymatic hydrolysis, which impact conversion to sugar positively compared to shorter plants.

These results are promising as they indicate that it should be possible to identify wheat cultivars that are suitable for dual purpose use (grain for food, straw for bioethanol) and that these are not likely to be more susceptible to lodging. There was some variation in straw digestibility between

cultivars but there was no evidence that higher digestibility leads to weaker stem and subsequently increases lodging risk. PGRs reduced plant height and therefore lodging risk without reducing straw yield so had no impact on potential bioethanol yield.

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