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The potential of triticale as a low input cereal for bioethanol production

by

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Abbreviations used

AA	Amino acid
AY	Alcohol yield
CO ₂	Carbon dioxide
DDGS	Dried distillers grains and solubles
DM	Dry matter
GHG	Greenhouse gas
GJ	Gigajoule
ha	Hectare
L	Litre
Ν	Nitrogen
NIAB	National Institute of Agricultural Botany
RL	Recommended List
SKCS	Single Kernel Characterisation System
SU	Saaten Union
SWRI	Scotch Whisky Research Institute
t	Metric Tonne
TGW	Thousand grain weight

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1 Abstract

The aim of this work was to quantify the performance of modern triticale varieties grown under UK conditions, to assess their value for the bioethanol market, and to evaluate any potential greenhouse gas savings made in comparison with production of winter wheat. Using twenty samples of winter triticale from UK Descriptive List trials harvested in 2007 (representing thirteen different varieties), alcohol yields (AY; L ethanol/t grain) were quantified as well as starch and protein contents, grain size and hardness. AY was determined based on a modified method for assessment of distilling wheats for the Recommended List, and compared to AY of two Istabraq (wheat) samples of similar protein contents. Triticale was shown to be a feedstock with high potential for bioethanol production, with a soft grain, giving alcohol yields comparable with Istabraq at equivalent grain protein contents (average 436 L/t DM at 11.5%grain protein). Some triticale varieties (Fidelio, SW Fargo, Trimester, Ego and Grenado in particular) showed better than expected AY based on their protein contents, thus demonstrating their potential as feedstocks for bioethanol production. The ratio of conversion of starch to alcohol (6.44 L/10 kg starch) was relatively high compared to values seen previously for wheat. Further work is needed to understand variation in starch and fermentable sugars in modern triticale varieties, and in different agronomic situations. Residue viscosity of the triticale samples was higher than that of wheat, but alcohol yields should now be assessed using industrial enzymes representative of those which would be used in a modern bioethanol plant and which would reduce viscosity. In all scenarios studied using the HGCA Biofuels calculator, the net benefits in terms of reducing GHG emissions associated with bioethanol production, were greater for triticale than for wheat, principally due the lower N requirement of triticale. However, there is considerable uncertainty regarding the average grain protein content of triticale when fertilized at the economic optimum. There is also no data on the relative yields of wheat and triticale grown with their respective N optima, in both high and low yield potential situations. The major advantage of triticale may be in 2nd/3rd cereal positions in the rotation when the yield of wheat tends to be reduced by take-all. More work needs to be carried out to compare these two species side by side in replicated trials.

2 Project Summary

2.2 Objectives of the study

The objectives of this study were to quantify the performance and alcohol processing yield of modern triticale varieties; to assess the residue viscosity of triticale fermented at lab scale and compare to that of wheat; and to evaluate the potential greenhouse gas (GHG) savings of UK grown triticale compared to other UK cereal species.

2.2 Background

Biofuels such as bioethanol provide a renewable alternative to fossil fuels and an opportunity to reduce GHG emissions associated with energy use. In Sweden, both wheat and triticale (a hybrid of rye and wheat) are used for bioethanol production. Triticale has a number of potential advantages as a feedstock due to its lower nitrogen (N) requirement during crop growth, its ability to out-yield wheat in some situations, particularly on light soils, and in 2nd/3rd cereal positions in the rotation because of its better take-all resistance. However in UK agriculture, triticale has been undervalued in recent years, yet with N input costs rising and an interest in new markets such as biofuels, it is timely to revisit triticale as an alternative low-cost cereal.

Triticale has a soft grain; therefore its texture resembles more the soft wheats currently preferred by the distilling industry, than hard bread making varieties of wheat. The lower N requirement of triticale will be of great benefit if an accreditation scheme for bioethanol production sets tighter targets in the future with respect to the benefits which should be achieved in terms of minimising GHG emissions. The aim of the present study was firstly to quantify the alcohol yields (AY) of triticale compared to a good distilling wheat, and secondly to estimate the potential benefits from using triticale in order to maximise the net benefits from reducing GHG emissions associated with bioethanol production.

2.3 Materials and methods

Twenty samples of triticale representing thirteen different varieties were sourced from Recommended List trials at 2007 harvest (two sites). Grain size, hardness, starch, and protein content and AY were measured. Alcohol yield was also determined for two samples of Istabraq winter wheat taken from a nitrogen response experiment, for

comparison. Modelling of the net benefits from reducing GHG emissions associated with bioethanol production from wheat and triticale was carried out using the HGCA Biofuels calculator, by varying grain yields and N inputs for three different scenarios (effects of disease control, low yield potential and place in rotation).

2.4 Results and Discussion

2.4.1 Grain quality of triticale and its potential for bioethanol production

The triticale samples showed wide variation in grain size and protein content between varieties and sites, characters which are known to influence alcohol yield in wheat. As expected the site which produced the highest grain protein contents also had the lowest alcohol yields, and as seen with wheat, hardness increased as grain protein increased. The triticale samples studied gave alcohol yields comparable with Istabraq at equivalent grain protein contents (average 436 L/t DM at 11.5% grain protein). Some triticale varieties (Fidelio, SW Fargo, Trimester, Ego and Grenado in particular) showed better than expected AY based on their protein contents, thus demonstrating their potential as feedstocks for bioethanol production. The ratio of conversion of starch to alcohol (6.44 L/10 kg starch) was relatively high compared to values seen previously for wheat. Further work is needed to understand variation in starch and fermentable sugars in modern triticale samples was higher than that of wheat, but alcohol yields should now be assessed using industrial enzymes representative of those which would be used in a modern bioethanol plant and which would reduce viscosity.

2.4.2 Benefits of triticale in reducing GHG emissions associated with biofuel production

The outputs of the Biofuels calculator are reported in terms of a percentage reduction in emissions (of CO_2 equivalents) associated with bioethanol production, relative to petrol on a per GJ basis. The results show that both fungicide treated and untreated triticale show greater benefits in terms of reduced GHG emissions (35.6% & 30.7% respectively) than wheat (25.8 & 10.7% for treated and untreated respectively). The better performance of triticale is due to its lower N inputs. The better performance of the treated crops is due to the higher grain yields when diseases are controlled, which reduce the intensity of GHG emissions per tonne of bioethanol produced. Considering low yield scenarios on a typical sand land site, the net benefits of growing triticale and wheat for bioethanol were similar (17.8 and 16.4% respectively). Using a slightly higher yield estimate taken from Nix ('low production level' for both species), the triticale shows a better reduction in net GHG emissions (32.8%) compared to wheat (22.8%).

Considering place in the rotation, triticale shows greater benefits in terms of reduced GHG emissions compared to wheat, in both 1^{st} and 2^{nd} cereal positions: A reduction in yield of 1 t/ha for wheat and slightly increased N fertiliser (+20 kgN/ha) applied to a second wheat reduces the net benefits of bioethanol production from 25.8 to 12.5%. In contrast, the benefits from growing triticale only reduce from 36.1 to 32.8%, associated with a loss of yield of 0.4 t/ha in the 2^{nd} cereal position and no change in N inputs. The benefit from wheat would be even smaller if a larger yield loss was assumed e.g. in a high take-all situation.

2.5 Key conclusions

- Triticale is a feedstock with high potential for bioethanol production, giving alcohol yields per tonne of grain comparable with a good distilling wheat (Istabraq) at equivalent grain protein content.
- 2. In all scenarios studied using the HGCA Biofuels calculator, the net benefits in terms of reducing GHG emissions associated with bioethanol production were greater for triticale than for wheat, principally due the lower N requirement of triticale.
- 3. Some triticale varieties showed better than expected alcohol yields (L/t) based on their starch and protein contents and further work is needed to understand variation in starch and fermentable sugars in modern triticale varieties, and in different agronomic situations.
- 4. The Scotch whisky lab method for spirit yield was used here to ensure comparison with existing RL data for wheat, but alcohol yields should also be assessed using industrial enzymes representative of those which would be used in a modern bioethanol plant, particularly in terms of controlling viscosity.

- 5. There is considerable uncertainty regarding the average grain protein content of triticale when fertilized at the economic optimum, and further work needs to be carried out in trials where wheat and triticale are grown alongside one another.
- 6. The major advantage of triticale may be in 2nd/3rd cereal positions in the rotation when the yield of wheat tends to be reduced by take-all and more work needs to be carried out to compare these two species side by side in replicated trials.

3 Introduction

Biofuels provide a renewable alternative to fossil fuels and an opportunity to reduce greenhouse gas (GHG) emissions associated with energy use. Based on the available feedstocks at the present time, the biofuel with potentially the largest volume in Northern Europe is bioethanol produced from the fermentation of sugars derived from starchy cereal grains. Different sources of grain are used around the world for bioethanol production, with the USA using maize; Germany and Poland, wheat and rye; and Sweden, wheat and triticale. Processing on a large scale consists of mashing, fermenting and distilling using methods currently employed by the brewing and distillery industries. Several plants are currently being planned around the UK as of this year, most of which are currently designed to use wheat as it provides more harvestable starch than any other crop in the UK (Smith *et al.* 2006) which it does broadly speaking with high levels of nitrogen (N) inputs.

Production of bioethanol globally is led by the United States, Brazil and China. However in Europe, bioethanol production is growing rapidly, with Germany leading and producing 70% of its ethanol requirements. In 2001, Sweden opened its first bioethanol plant, and Agroetanol is reported to be researching a feedstock blend including 40% triticale. In Europe, Sweden also has the highest number of bioethanol fuel stations (at 792 in comparison with the UK's 14) where it is compulsory by law for each station to have at least one alternative fuel (Johansen, 2007). The predicted demand for the UK is equivalent to 2.5 million tonnes of wheat in 2010 (Smith *et al.* 2006). Whilst in the short term these needs can be met with importation, being able to increase efficiency in crop rotation with alternative cereals could be highly advantageous for the UK industry.

Additionally, with a new market being created and a finite land resource, it is likely that more second and third wheats will be grown, with a resulting decline in yield in those crops due to take-all. It is therefore timely to review other cereals particularly for marginal (e.g. acid, light soils) and for 2nd and 3rd positions in the cereal rotation which have a resistance to take-all, but which can also meet the biofuels producers or distillers needs. This report explores the opportunities for triticale as a cereal which can provide benefits in such scenarios.

3.1 History of triticale

Triticale is a hybrid of rye and wheat, and can be present both in octaploid forms (AABBDDRR) and hexaploid types (AABBRR). In general it is thought that the hexaploid forms (mainly durum wheat x rye) appear to be more useful (Gill & Vear, 1980). At the time of writing this report, it is not clear what the ploidy levels of current commercial varieties are, although this would be straightforward to check if necessary. Hybrids between wheat and rye were first reported in 1875 but triticale varieties were only released commercially in 1969. During the 1970's breeders began to improve and release the early types, and by the early 1980s, various groups in the UK had begun to trial triticale (Naylor, 1987a, b; Aquilina, 1987). One of the main advantages of triticale is that it has much better take-all resistance than wheat (Hollins *et al.*, 1986) halfway between that of wheat (susceptible) and rye (resistant).

With the yield potential of wheat in 2nd/3rd cereal positions or on light land, and the hardiness of rye, triticale has been widely cultivated around the world, being successfully grown almost anywhere its parent species are grown (Varughese *et al.*, 1997). In 2005, 13.5 million tonnes were harvested globally (FAO). However since the 1980's in the UK, more rapid yield improvements in wheat mean that triticale has generally been outclassed. Therefore the early interest in triticale has not been maintained. The reliance on RL yield data (in predominantly 1st cereal positions for wheat) and lower gross margins reported for triticale by Nix (2007) means that triticale is currently seen as being of little value in UK agriculture, and excepting the data of Overthrow and Carver (2003) much of the reported trial data is 25 years out of date. Its advantages are discussed further below.

3.2 Agronomic advantages of triticale

Position in rotation

Triticale shows many agronomic advantages including tolerance of acid soils, light soils and dry conditions. ADAS trials in the 1980's demonstrated that triticale varieties

could out-yield wheat in the UK in the second cereal position by 1.88 t/ha on a light organic soil (Cleal, 1993) although this was using the older varieties Galahad (wheat) and Cumulus, Lasko and Purdy (triticale). The yield of Galahad in these trials was 53% of the reported RL yield at the time, whereas the average yield of the triticale varieties was 99% of the RL control yields for triticale on light soils (NIAB, 1986). Earlier ADAS data on the same light soil (Anon, 1984) indicated that take–all affected 56% of triticale plants and 7% of the roots, whereas it affected 90% of wheat plants and 30% of roots (variety Avalon) in a 2nd cereal position.

More recent UK research has also shown that triticale can be a better option than wheat on marginal land or as a 2^{nd} or 3^{rd} position cereal yielding as much as 8 t/ha in plot yields (Overthrow and Carver, 2003).

3.2.1 Nitrogen nutrition

When considering grain for alcohol production, nitrogen fertiliser inputs are important because of their effect on grain protein, increasing it and thereby reducing starch content and alcohol yield. Early reports suggested that grain protein content in triticale was higher than that in wheat (Gill and Vear, 1980) but this may be partly due to the relatively lower yields of early triticale releases compared to wheat (proteins being effectively diluted in high yielding wheats).

This observation was apparently confirmed in Scotland by Naylor (1987b) who compared Longbow (wheat) and Lasko (triticale) over a range of N rates from 0 to 180 kgN/ha. He found the triticale to have a grain protein content 2.8% higher than wheat at the highest N rate applied. However, from a current perspective, these data are unsatisfactory because the wheat may have been under fertilised (the highest grain protein for wheat in that trial was only 7.2%), while the highest N rate applied to the triticale would be above the recommended optimum. To further underline the lack of sound data around N responses of triticale, in 1983 a series of N response trials were carried out at seven ADAS sites using triticale line WTCB 134 with and without growth regulator (Anon, 1984), but unfortunately grain protein data from these trials was not published.

Overthrow and Carver (2003) showed very small differences in grain protein contents between rye and wheat in the $2^{nd}/3^{rd}$ cereal position (wheat 0.8% lower than triticale

at Cirencester and 0.18% higher at the Caythorpe site). The N applied in these trials was not reported: If it is assumed that both cereals received a uniform rate, then effectively the wheat may have been under fertilised (average grain protein for wheat was only 10.79% in these trials). In reality a second wheat grower may increase the fertiliser N applied to account for the lower N uptake anticipated through poorer rooting. It is concluded that there do not appear to be any data on comparable N responses for modern wheat and triticale varieties, grown side by side. Triticale already has a lower N requirement as stated in the fertiliser recommendations (RB209; Anon, 2000) where the maximum application allowed is stated at 130 kgN/ha. This low input compared to wheat (typically 220 kgN/ha at N index 1 on a similar soil type) not only reduces the economic cost of production but presents an opportunity to reduce greenhouse gas emissions (Kindred et al. 2007a). Minimising the GHG emissions associated with crop production will be critical to achieving sustainable biofuel production. This will be of increased importance if there is a rigorous accreditation scheme in place, or there are financial rewards for growers producing grain for alcohol with an associated reduction in GHG emissions (Sylvester-Bradley & Kindred, 2008).

3.3 Alcohol yield and processing benefits of triticale

Above and beyond the agronomic benefits and potentially lower grain protein contents, triticale is perceived to have grain quality advantages that make it beneficial for fuel alcohol production, namely higher auto-amylolytic activity than other cereals (including wheat and rye). Thus triticale has been reported as being used without the addition of enzymes, reducing the consumption of enzyme preparations by up to 50% (Kučerová, 2007).

Earlier research has been somewhat conflicting with published data from Rosenberger (2005) finding that triticale gave less alcohol per unit of starch than both wheat and rye. In contrast, higher alcohol yields were recorded from triticale compared to wheat by Fleischer and Senn (2005) and Aufhammer *et al.* (1994). It is highly likely that these contradictory results are partly due to differences in protein content not being taken into account. Therefore there is a need to assess triticale on a 'like for like'

basis, taking into account recent knowledge of grain size, shape and protein content from wheat (Kindred et al. 2007b).

3.4 Viscosity of triticale

Rye contains higher concentrations of arabinoxylans (or 'pentosans') than wheat which contribute to higher viscosity when rye is mashed. Historically triticale varieties with a higher complement of rye chromosomes were expected to give high viscosities compared to bread wheat. This is analogous to the way some 1B1R wheats tend to have higher viscosities (Weightman *et al.*, 2001) both for distilling and in an animal feed context due partly to their higher arabinoxylan content (Dhaliwal and MacRitchie, 1990). However, some modern triticale varieties can give low viscosities similar to that of the traditional soft wheats. It is important therefore, to determine the residue viscosities of the modern triticale varieties to assess their suitability for the production of alcohol.

Since none of the wheat varieties which have currently received a distilling recommendation on the RL possess the 1B1R translocation, the problem of viscosity is important from a biofuels perspective. However it should be noted that in a biofuels plant, there is an option to use enzymes to control viscosity (both for triticale and wheat) which is not available to whisky producers. Therefore while there is a cost of additional enzymes, the technical hurdles to deal with viscosity in a biofuels plant are not great. Clearly further information is needed to quantify viscosity of modern triticale varieties and comment on their importance relative to wheat.

3.6 Feeding value of DDGS from triticale

Another potential benefit of triticale is its nutritional value in terms of the amino acid (AA) composition of the distillers dried grains and solubles (DDGS) as a feed for monogastric animals. A number of others have reported that triticale has a higher lysine content than wheat (Lásztity, 1984; Oelke *et al.*, 1989). Based on typical AA concentrations of wheat and triticale grain (Lásztity, 1984) and protein contents of 10.5 and 11.5 % for triticale and wheat respectively, and assuming that the protein content of DDGS is increased 3.5X above that in the grain (Cottrill *et al.*, 2007), the estimated AA composition of the DDGS is shown in Table 1.

<u>Composition</u>						
Amino acid	Protein basis (g/100g protein)			DDGS basis (g/100g DDGS)		
	Triticale ¹	\mathbf{Wheat}^1	Triticale (estimated)	Wheat (estimated)	Wheat Ref ²	
Lysine	2.80	2.10	1.03	0.85	0.7	
Histidine	2.34	2.31	0.86	0.93	0.7	
Arginine	4.77	3.67	1.75	1.48	1.4	
Aspartic acid	5.67	3.43	2.08	1.38	1.6	
Threonine	3.05	2.51	1.12	1.01	1.0	
Serine	4.37	4.07	1.61	1.64	1.5	
Glutamic acid	32.91	40.53	12.09	16.31	8.2	
Proline	14.18	12.54	5.21	5.05	nd	
Glycine	3.87	3.60	1.42	1.45	1.3	
Alanine	3.55	2.88	1.30	1.16	1.2	
Cystine	3.22	2.49	1.18	1.00	0.6	
Valine	4.93	4.34	1.81	1.75	1.4	
Methionine	2.25	1.70	0.83	0.68	0.5	
Isoleucine	4.37	3.94	1.61	1.59	1.1	
Leucine	7.55	7.11	2.77	2.86	2.1	
Tyrosine	2.81	2.48	1.03	1.00	0.9	
Phenylalanine	4.98	6.09	1.83	2.45	1.4	
Tryptophan	nd	Nd	-	-	0.4	

 Table 1. Amino acid composition of triticale and wheat, and their respective forms of DDGS assuming concentration of protein by 3.5X during distilling

¹, Data from Lásztity (1984)

², French data from Vilarino (2006)

No actual data were found on the AA composition of triticale DDGS and these estimates must be treated with some reservations: There is considerable variation in individual AA composition between different sources of wheat DDGS (Cottrill et al. 2007), which will partly be due to variation in the starting feedstock, but also due to losses of certain AA, particularly lysine during processing. The yeast will also contribute some AA, not accounted for here.

3.6 Aim of the project

The aim of this project was to study the alcohol yields and viscosities of a range of triticale varieties using the method currently used to score distilling wheats for the Recommended List. It was not possible within the resources available to study in detail factors such as N nutrition or place in the rotation on grain characteristics. However, samples were supplied from two contrasting sites which did differ in level of

N nutrition, which meant a wider range of variation (environmental as well as genotypic) was incorporated. Comparisons were made with two samples of Istabraq (wheat) contrasting in grain protein levels, as Istabraq is currently recommended for distilling on the RL. Finally, based on typical yields and grain protein contents for the two cereal types, the relative greenhouse gas emissions per tonne of bioethanol produced were modelled using the HGCA Biofuels calculator.

4 Materials and Methods

4.1 Samples

Twenty triticale samples were supplied by Senova UK Ltd representing 13 varieties from two Recommended List trials sites harvested in 2007 (Table 2; trials managed by NIAB and Saaten Union (SU)). Trial samples supplied to ADAS were unreplicated. Pedigrees of the triticale varieties where known are shown in Table 3.

A higher rate of applied N was used at the SU site (190 kgN/ha), compared to the NIAB site (128 kgN/ha). Grain yields were supplied by site managers. Samples TRIT-4, 7, 10, 13, and 14 contained some ergot sclerotia but in all cases this was less that 1 ergot per kg of grain where found. Samples 1 and 11 showed evidence of sprouted grain. In addition, two samples of Istabraq winter wheat were analysed, in order to compare the triticale samples with a 'good' distilling wheat.

		Site	
Sample ID	Variety	code	Full description
Saaten Union site			
TRIT-1	Puerto	1	Puerto Saaten-Union 215-10A
TRIT-2	Taurus	1	Taurus Saaten-Union 215-13A
TRIT-3	SW Valentino	1	SW Valentino Saaten-Union 215-11A
TRIT-4	Grenado	1	Grenado Saaten-Union 215-9B
TRIT-5	SW Fargo	1	SW Fargo Saaten-Union 215-12A
TRIT-6	Ego	1	Ego Saaten-Union 215-7A
TRIT-7	Fidelio	1	Fidelio Saaten-Union 215-8A
TRIT-8	Benetto	1	Benetto Saaten-Union 215-6A
NIAB site			
TRIT-9	Fidelio	2	Fidelio NIAB
TRIT-10	Grenado	2	Grenado NIAB
TRIT-11	Puerto	2	Puerto NIAB
TRIT-12	Ego	2	Ego NIAB
TRIT-13	SW Fargo	2	SW Fargo NIAB
TRIT-14	Trigold	2	Trigold NIAB (LP5699)
TRIT-15	Trimester	2	Trimester NIAB
TRIT-16	Borwo	2	Borwo NIAB (BOH 504)
TRIT-17	SW Valentino	2	SW Valentino NIAB
TRIT-18	Gringo	2	Gringo NIAB (DED 650/1)
TRIT-19	Benetto	2	Benetto NIAB
TRIT-20	Kasyno	2	Kasyno NIAB (DED 187/00)
WHEAT-1	Istabraq		High protein samples from wheat N response experiment*
WHEAT-2	Istabraq		Low protein sample sourced as above*

Table 2. Varieties and sites in 2007 used to supply triticale samples

* HGCA project no RD-2004-3084

Variety	Pedigree (where known)
Puerto	Fidelio x Ego
Taurus	Salva x CHD 777/81
SW Valentino	Holme /Kustro//IA-Beagle/3/247-320/Beagle
Grenado	(LA85/90 x Presto) x Chrono
SW Fargo	Fidelio x (Ego x Fidelio)
Ego	Alamo x (Dagro x Bezostaja)
Fidelio	[(Lanca wheat x rye L506/79) x L 627/80 trit] x CT 776/81 trit
Benetto	(CT932.89 x CHD510.86) x Moreno
Trigold	LP 10009.93 x LP 9875.4.94
Trimester	(Trimaran x MT16482-1) x Binova
Borwo	MAH 15841-13 x LAD 794
Gringo	(DTK 574/94 x DAD 275/94) x Woltario
Kasyno	Information not available

Table 3. Pedigrees of triticale varieties studied

4.2 Analytical methods

Proximate analysis

Protein was estimated as Nx5.7, following determination of grain N content by Dumas combustion. Starch was determined by the Ewers polarimetric method as described by Kindred *et al.* (2007b).

Grain size and texture

Mean grain weight (mg), width (mm) and hardness index were measured using the Single Kernel Characterisation System (SKCS). Thousand grain weight was then estimated as 1000 x mean grain weight.

Alcohol yield determination

Triticale grain was milled using a Glen Creston hammer mill fitted with a 2 mm screen, and the moisture content of the flour determined on a subsample by drying overnight at 100 °C. Alcohol yield and viscosity were determined in duplicate using an ADAS method adapted from that of the Scotch Whisky Research Institute (SWRI; Agu *et al.*, 2006) as follows: Wholemeal flour (15 g fresh weight basis) was placed in a stainless steel beaker with 40.5 mL of water and 250 ųL of a thermostable alpha-amylase (added in excess) to rapidly break down starch to oligosaccharides (Termamyl 120L, Novozyme). The slurry was then heated in a waterbath to 85 °C with frequent stirring, before being autoclaved at 126 °C for 11 min. The sample was returned to the waterbath and further 250 µl of the amylase was added when the slurry returned to 85 °C, to minimise retrogradation. The cooked slurry was then reduced in temperature and mashed at 65°C for an hour with inclusion of barley malt that contains a relatively high a and β amylase content and also supplies modified starch and free amino nitrogen to the yeast (20% malt to 80% wheat on a dry weight basis). The slurry was pitched with distillers yeast (0.4% w/w) and fermented at 30°C for 68 hours before being distilled and the distillate measured for alcohol content using an Anton Paar density meter. The residue after distillation was adjusted to 125 mL with water before being centrifuged and the supernatant filtered twice through GF/A filter papers. Viscosity of the supernatant was determined at 20 °C using a U-tube viscometer (PSL-BS/U B, Poulten Selfe & Lee, Essex, UK).

4.3 Assessment of greenhouse gas emissions associated with various production scenarios

The impact of various scenarios on the net benefits of bioethanol production from triticale compared to wheat was assessed using the HGCA Biofuels calculator (v1.1 g, accessed 23/6/08). The 'wheat to bioethanol' option was used whereby triticale was simply treated as wheat, with the exception that in the processing function, triticale and wheat were assumed to have alcohol yields of 376 and 369 L/t @85% DM (442 and 434 L/t at 100% DM; see note 1) respectively when fertilised at their economic optima.

Changes were then made to inputs of N fertilizer (kg/ha) and to farm grain yield (t/ha @85%DM; see note 2) for each cereal in the different agronomic scenarios (Annexes B-D). Otherwise all other inputs remained the same as shown in Annex A.

The Biofuels calculator estimates the total CO_2 equivalent (CO_2 eq.) associated with each tonne of bioethanol produced using the various agronomic and processing scenarios, and then compares this to the CO_2 eq. emitted from using the same amount of energy from petrol. As an example, triticale output at 6.5 t/ha and with 130 kg N/ha inputs generates 1524 kg CO_2 eq./t bioethanol, and this equates to 56.6 kg CO_2 eq./GJ of energy produced. Petrol generates 87.4 kg CO_2 eq./GJ energy, therefore the production of bioethanol 'saves' 87.4 - 56.6 = 30.8 kg CO_2 eq./GJ compared to petrol. This equates to a reduction in emissions relative to petrol of 35% (=30.8/87.4 × 100).

Notes:

(1) AY was estimated from the predictive equation for wheat as follows:
 AY (L/t, DM basis) = -7.31 x protein +519 [from Smith *et al.*, 2006].

This equation has been applied to both wheat and triticale in the modelling exercise as it is built from a much bigger dataset than was generated using the limited number of triticale samples studied in this project. Therefore it is more widely applicable to the general modelling scenarios.

(2) The inputs to the biofuels calculator have been based on farm yields which are generally lower than experimental plot yields (because of the lower yields associated with headlands and tramlines). In order to model different scenarios where data were taken from field experiments (e.g. the effects of fungicide treatment) the equivalent farm yields were estimated as follows:

Farm yield = experimental plot yield x 0.8

5 Results

As the grain samples received were unreplicated within a site, it was not possible to make statistical comparisons between varieties. Site means could be compared using a t test at p=0.05. Care should be taken in interpreting these results as not all varieties are represented at each site. Therefore statistical significance of site effects reported should be treated as indicative only.

Grain yield was significantly higher at the SU site for the common varieties (Table 4), reflecting the higher level of nutrition at that site. TGW and grain diameter were also greater at the SU site. Fidelio at the SU site had the largest grain size.

Sample ID	Variety	Yield (t/ha @ 85% DM)	TGW (g)	Diameter (mm)	Hardness (SKCS)
Saaten Union site					_ <u> </u>
TRIT-1	Puerto	8.34	49.0	3.19	55.5
TRIT-2	Taurus	7.82	38.4	2.71	53.6
TRIT-3	SW Valentino	8.89	41.8	2.76	60.1
TRIT-4	Grenado	8.61	41.6	2.65	54.0
TRIT-5	SW Fargo	8.42	47.4	3.09	59.4
TRIT-6	Ego	8.46	42.4	2.85	63.6
TRIT-7	Fidelio	8.33	52.7	3.25	55.9
TRIT-8	Benetto	8.89	50.6	3.00	57.9
	Overall site mean:	8.47	45.5	2.94	57.5
	Common variety mean†:	8.56	46.5	2.97	58.0
	Common variety SE:	0.091	1.72	0.085	1.24
<u>NIAB site</u>					
TRIT-9	Fidelio	6.56	42.7	2.89	49.6
TRIT-10	Grenado	8.40	37.0	2.51	39.5
TRIT-11	Puerto	7.63	48.0	3.08	53.4
TRIT-12	Ego	7.37	43.2	2.87	58.8
TRIT-13	SW Fargo	7.19	45.0	2.97	56.9
TRIT-14	Trigold	7.66	35.2	2.60	34.1
TRIT-15	Trimester	7.03	44.6	2.73	30.2
TRIT-16	Borwo	8.62	43.6	2.92	58.5
TRIT-17	SW Valentino	6.59	35.8	2.49	49.5
TRIT-18	Gringo	8.11	42.7	2.78	43.8
TRIT-19	Benetto	6.58	33.8	2.36	49.3
TRIT-20	Kasyno	6.41	30.8	2.22	54.8
	Overall site mean:	7.35	40.2	2.70	48.2
	Common variety mean:	7.19	40.8	2.74	51.0
	Common variety SE:	0.259	2.00	0.106	2.40
Sig. (of site effect for common varieties:	*	NS	NS	NS

Table 4. Grain yield, grain size and texture for 20 samples of triticaleharvested from two RL sites in 2007

⁺, Common varieties; Puerto, SW Valentino, Grenado, SW Fargo, Ego, Fidelio, Benetto.

*, Sig at p=0.05; NS, not significant

Hardness was also greater at the SU site, which may reflect the significantly higher grain proteins seen at that site (Table 5). In contrast with grain protein, the starch concentrations were significantly higher at the NIAB site and this was reflected in higher alcohol yields (Table 5). For the common varieties, alcohol yield: starch ratio and residue viscosity were not significantly different between the two sites (based on a t-test at p=0.05).

Sample	Variety	Protein (g/100g)	Starch (g/100g)	AY (L/t)	AY:starch (L/10kg)	Viscosity (mPa s)
Saaten Unio	on site					
TRIT-1	Puerto	13.64	63.2	404	6.39	2.50
TRIT-2	Taurus	13.01	70.0	419	5.98	2.42
TRIT-3	SW Valentino	12.22	66.9	420	6.29	2.40
TRIT-4	Grenado	13.11	67.8	416	6.14	2.52
TRIT-5	SW Fargo	12.42	66.3	433	6.54	2.11
TRIT-6	Ego	13.08	67.2	444	6.60	2.48
TRIT-7	Fidelio	13.48	65.5	435	6.64	2.41
TRIT-8	Benetto	12.60	66.2	423	6.39	2.46
	Overall site mean:	12.94	66.6	424	6.37	2.41
Comm	on variety mean†:	12.93	66.2	425	6.43	2.41
Con	nmon varieties SE:	0.204	0.56	5.0	0.069	0.053
<u>NIAB site</u>						
TRIT-9	Fidelio	10.67	68.9	450	6.52	2.72
TRIT-10	Grenado	9.85	70.3	465	6.61	2.46
TRIT-11	Puerto	10.77	68.8	437	6.35	2.18
TRIT-12	Ego	10.69	68.5	437	6.38	2.32
TRIT-13	SW Fargo	10.77	68.6	458	6.67	3.01
TRIT-14	Trigold	10.15	68.1	450	6.60	2.23
TRIT-15	Trimester	9.78	68.1	461	6.77	2.07
TRIT-16	Borwo	10.23	70.3	449	6.38	2.61
TRIT-17	SW Valentino	10.64	67.3	423	6.29	2.19
TRIT-18	Gringo	10.77	68.2	443	6.50	2.31
TRIT-19	Benetto	10.39	67.1	441	6.57	3.23
TRIT-20	Kasyno	11.20	68.2	416	6.09	3.53
	Overall site mean:	10.49	68.5	444	6.48	2.57
Comi	mon variety mean:	10.54	68.5	444	6.48	2.59
	nmon varieties SE:	0.124	0.41	5.3	0.055	0.156
	<i>ig. of site effect for common varieties:</i>	*	*	NS	NS	NS

Table 5. Grain protein and starch concentration, alcohol yield (AY) on a
100% DM basis, and extract viscosity of 20 triticale samples harvested from
two RL sites in 2007

⁺, Common varieties; Puerto, SW Valentino, Grenado, SW Fargo, Ego, Fidelio, Benetto.

*, Sig at p=0.05; NS, not significant

A plot of alcohol yield against grain protein (Figure 1) shows that there was a negative relationship between the two grain characters, and a simple linear regression

indicated that variation in protein content explained 50% of the variation in alcohol yield. The two samples of Istabraq (a good distilling wheat; Table 6) fell in the middle of this relationship, indicating that on average the triticale varieties are behaving like good distilling wheats in terms of their potential alcohol yield.

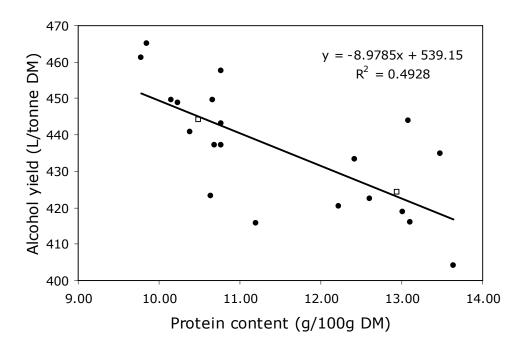


Fig 1. Relationship between alcohol yield and protein concentration for twenty samples of triticale (\bullet) and two samples of wheat (\Box , variety Istabraq)

The predictive equation from Fig. 1 could also be used to assess the relative performance of varieties, by comparing actual alcohol yields at a given protein content, to those predicted using the regression equation. The analysis shows that Fidelio, SW Fargo, Ego, Grenado, Trigold, Borwo and Gringo all giving more alcohol (L/t) than would be expected based on their protein contents (Table 7). In particular Fidelio and SW Fargo appeared to be the superior varieties. The relative positions of the varieties are also demonstrated visually in Figure 2.

Table 6. Grain protein concentration, measured alcohol yield (AY), predicted alcohol yield (based on equation in Fig.1) and residue viscosity for two reference samples of Istabraq winter wheat of contrasting protein contents

Sample	Protein (g/100g)	Measured AY (L/t)	Predicted AY* (L/t)	Viscosity (mPa s)
Istabraq – 1	10.55	451	444	1.68
Istabraq – 2	12.71	416	425	1.66
Mean:	11.63	434	435	1.67

* predicted from equation AY=-8.9785 x protein + 539 (see Fig 1)

There was also a relationship between starch and protein content (simple linear regression equation: Starch =-0.8052 x protein +77, figure not shown) but this was much poorer than the relationship between AY and protein ($R^2 = 0.3$). Again, Fidelio, SW Fargo and Trimester showed higher than predicted levels of starch, which support their tendency for higher alcohol yields (Table 5). However, Benetto and Puerto also gave higher than predicted starch contents, but lower than predicted alcohol yields.

Table 7. Deviation of measured alcohol yield and starch concentration from the predicted, for 13 varieties of triticale (based on the relationship between each trait and grain protein content)

Variety	No. of observations	AY deviation * (%)	Starch deviation † (%)
Fidelio	2	2.71	0.11
SW Fargo	2	2.37	0.25
Trimester	1	2.18	1.02
Ego	2	1.96	0.43
Grenado	2	0.93	-1.29
Trigold	1	0.33	0.77
Borwo	1	0.31	-1.57
Gringo	1	0.12	0.19
Taurus	1	-0.85	-3.48
Benetto	2	-0.97	1.12
Puerto	2	-2.10	1.14
SW Valentino	2	-3.34	0.75
Kasyno	1	-5.21	-0.23

*, predicted from equation AY=-8.9785 x protein + 539 (see Fig 1)

+, predicted from equation Starch =-0.8052 x protein +77 (not shown)

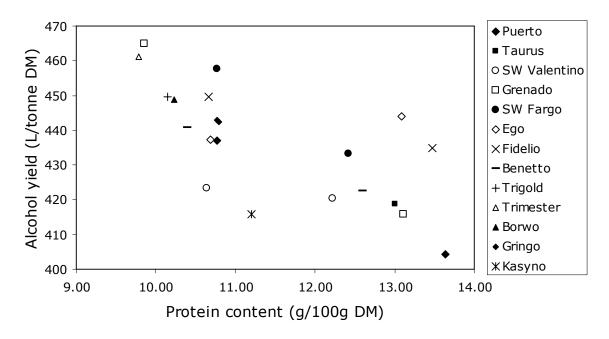


Fig 2. Relationship between alcohol yield and protein concentration for thirteen varieties of triticale harvested in 2007.

6 Discussion

6.1 Grain characters and determination of alcohol yield

This is the first UK study to consider grain quality characters of triticale and to consider the potential of triticale for the biofuel and distilling markets. The variation seen in grain size (TGW; 30.8 - 52.7 g) and diameter (2.22 - 3.25 mm) is typical for any small grain cereal and based on our knowledge of wheat, suggests that there will also be variation in alcohol yield between triticale varieties. For example it is known that some varieties of wheat e.g. Riband, achieve high alcohol yields through having large well-filled grains (Kindred *et al.* 2007b) and low length: width (L:W) ratios (Swanston *et al.*, 2007). Although L:W ratio was not measured in the present study, variation in grain shape was apparent, indicating that triticale needs to be considered in the same way as wheat when aiming to understand alcohol yields. Fidelio, which had the largest grain size (at the SU site), also showed some of the best potential alcohol yields.

Triticale was shown to have a relatively soft grain, with texture between a hard and soft wheat. This is important, as the distilling industry traditionally prefers soft wheats (and currently only soft wheat varieties are assessed for distilling on the RL). As seen with wheat, the lower protein samples (e.g. Grenado and Trimester), tended to be softer and the lower protein (NIAB) site also produced samples with a softer grain. Again, this indicates that in terms of grain quality parameters, the triticale samples were behaving essentially like wheat, where protein content and hardness are positively related.

Variation was also seen in grain protein in this study and this is known from wheat to be the major predictor of alcohol yield. Average grain protein at the NIAB site (10.5%) which had 128 kgN/ha applied, was slightly lower than would be expected for wheat grown at the economic optimum rate of N fertiliser (11.5%; Sylvester-Bradley personal comm.). In the absence of other information, these relative values for grain protein of triticale and wheat were taken forward into modelling GHG emissions using the Biofuels calculator (see below).

The best current predictor of high alcohol yield is low protein content and both variety and agronomy should be used to minimise grain protein in samples for biofuel production. It has been reported in the literature for wheat that grain size is positively related to alcohol yield in certain varieties (Swanston *et al.*, 2007). In the present study it was noted that the SU site with the higher average grain proteins, also gave the highest average TGW. Therefore this relationship, clearly, is not widely applicable; in particular it does not apply where agronomic rather than genetic factors influence grain size.

It is clear here that all the highest alcohol yielding samples had low grain proteins, and these came from the NIAB site with the lowest N inputs. The relationships between protein and alcohol yield are well documented in wheat (Riffkin *et al.*, 1990; Kindred *et al.*, 2007b) and it is demonstrated here that triticale behaves similarly. Although variation in grain protein only explained 50% of the variation in alcohol yield in the present study, it should be recognised that this is a small dataset (contrast with wheat where similar relationships using many years of CEL data give R² ~73%; Smith *et al.*, 2006). It was also shown that the alcohol yield for triticale was comparable with that of a good distilling wheat (Istabraq) at equivalent grain protein, and that these data correspond well with those of similar studies such as Fleischer and Senn (2005) and Aufhammer *et al.* (1994). The average alcohol yield obtained for all the triticale samples in the present study was 436 L/t (DM basis) with the average reported for wheat at 435 L/t (Smith *et al.*, 2006). However some of the triticale varieties give higher alcohol yields than the Istabraq samples, the three highest yielding being Grenado, SW Fargo and Trimester.

The starch content appeared relatively low (average 68.5% at the NIAB site) compared to wheat (e.g. 70.5% for Riband and Option with an average grain protein of 11.5%; Kindred *et al.*, 2007b). The industry view that triticale has higher starch content than wheat may simply be due to the fact that most triticale samples studied have been low protein and consequently high starch, but better comparisons are required of wheat and triticale grown side by side. As a result the AY: starch ratio for the triticale was relatively high compared to wheat (6.44 vs. 6.30 L/10 kg starch). One explanation for this is that the free sugar content of triticale grain is higher than wheat which might compensate for its lower starch content (fermentation of free sugars also contributes to alcohol yield). However it was not possible to measure

sugars in the present study. Further work is required to more fully characterise the fermentable sugars and grain characteristics of triticale.

The main grain character which might be deemed a negative trait in triticale was its viscosity: Residue viscosities for the triticale varieties were appreciably higher (average 2.51 mPa s) than those of the two Istabraq samples (1.67 mPa s). However in commercial practice, this viscosity could be dealt with by using enzymes to degrade the arabinoxylans. It is only a problem in the traditional Scotch whisky process, where it is prohibited to use enzymes in this way in order to reduce viscosity. Further work is required to assess the performance of triticale using commercial enzymes.

6.2 The benefits of triticale for bioethanol in minimising GHG emissions

Based on the above analysis which indicated that some triticale varieties have potential alcohol yields comparable with good distilling wheats, this information could be combined with typical levels of applied N (inputs), and grain yields (outputs) to compare the relative reduction in GHG emissions per unit of bioethanol produced for these species.

For the various scenarios examined one main assumption is made: That a 'typical' triticale crop fertilised with a rate of N at the economic optimum will achieve a grain protein content of 10.5%, whereas wheat at its respective economic optimum will achieve 11.5% grain protein. Clearly there is some uncertainty over the triticale value, because (as discussed above) historic data suggested that triticale had higher grain proteins than wheat, whereas Overthrow and Carver showed no overall difference. The figure of 10.5% is taken from the value at the NIAB site in 2007 studied in this report. The only way to get a consistent answer regarding the grain N at the economic optimum of triticale will be to carry out N response trials for wheat and modern triticale varieties alongside each other in the same field experiment and this must be a target for further work.

Despite the uncertainty over typical grain composition, the differences in alcohol yield between triticale and wheat used in the model (376 vs 369 L/t @85%DM at 10.5 and 11.5 % protein respectively) have a trivial effect on the outputs of the Biofuels calculator. For example, this difference of 7 L/t between triticale and wheat only makes a difference of a 1.2% to the reduction in GHG emissions (at a fixed level of N

inputs, and grain yield). In contrast a change in N inputs from 130 to 220 kgN/ha (at a fixed AY of 369 L/t and grain yield of 7.5 t/ha) makes a difference of a 13.7% to the reduction in emissions. Thus, N inputs are much more important drivers of sustainable biofuels production than alcohol yield per tonne of grain, and this is where the main benefits of triticale lie.

Based on these assumptions of typical alcohol yields and recommended N inputs from RB209, a number of scenarios were modelled using the Biofuels calculator, in order to compare the effects of different levels of inputs and yields to triticale and wheat. These scenarios were:

- a) moderate yield potential site with and without fungicide treatment,
- b) low yield potential sites
- c) place in rotation $(1^{st}/2^{nd} wheats)$

General note on presentation of figures

Since for any particular agronomic scenario or species comparison, the particular grain yields chosen here could be challenged, the outputs of the Biofuels calculator have been illustrated visually in Figs. 3-5 to allow some flexibility in interpretation: Using the change in reduction in GHG emissions in response to grain yield at fixed levels of N fertiliser applied, curves were fitted to produce 'iso-nitrogen' responses. The reader can then visually make adjustments to grain yield for a particular rate of N input, by moving along the relevant curve.

6.2.1 Fungicide treatment

Figure 3 shows the effect of a yield response to fungicide treatment on GHG balance for triticale and wheat. There are no published data directly comparing wheat and triticale alongside one another +/- fungicide treatment, therefore example yields were taken from different sources. The data sources and model outputs are listed in Annex B.

The results show that both fungicide treated and untreated triticale give greater benefits in terms of reduced GHG emissions, than wheat. Fig. 3 also shows, particularly in the case of wheat, the importance of using fungicide to achieve high grain yield, to effectively reduce the net GHG emissions per unit area of land (see also Berry *et al.*, Plant Pathology, in press).

6.2.2 Low site yield potential

Figure 4 shows the performance of triticale compared to wheat at low yield potential sites. Two scenarios were chosen – a 'general' scenario from Nix (2007) for low output examples of triticale and wheat, or from trial data taken from ADAS Gleadthorpe, a light sandy, drought-prone soil. The data sources and model outputs are listed in Annex C.

It can be seen that at the lowest yield potential sandland site (ADAS Gleadthorpe), the net benefits of growing triticale and wheat for bioethanol, were similar. Using the slightly higher yield estimate taken from Nix, the triticale shows a better reduction in net GHG emissions compared to wheat. However it should be noted that if a value of 130 kgN/ha was used for the inputs to the wheat (RB209 recommendation for winter wheat at N index 1 on light soils) the benefits for wheat would increase to 36.5% (reduction in GHG emissions relative to petrol). This underlines again the importance of low N inputs to maximising the benefits of biofuel production, and indicates that these scenarios need to be tested further with real N response data.

6.2.3 Effect of place in the rotation

Figure 5 shows the performance of triticale compared to wheat in 1st and 2nd cereal positions in the rotation. As there was no current data (excepting that of Overthrow and Carver, but their sites generally had very low take-all severity and are therefore less useful) the basic scenario assumed that wheat loses 1 t/ha of grain yield in moving from the 1st to 2nd cereal position (J. McVittie, pers. comm.) and that the grower might increase N application from 220 to 240 kgN/ha to compensate for poorer rooting. A loss of 1 t/ha is equivalent to a 12% loss of yield from an 8.4 t/ha farm crop. Since triticale is thought to have take-all resistance equivalent to half that of wheat, the yield loss in triticale in the 2nd cereal position was assumed to be 6%. Details of yields and inputs are given in Annex D.

Triticale shows greater benefits in terms of reduced GHG emissions compared to wheat in both 1st and 2nd cereal positions. The GHG benefit from growing triticale would be even greater if a larger yield loss from wheat was assumed (e.g. using the loss of 47% yield for Galahad reported by Cleal, 1993).

It can also be seen from Fig. 5 that at a rate of 240 kgN/ha applied, once grain yield drops below 6.5 t/ha then the benefits for wheat would disappear (i.e. the % reduction in emissions become negative in value). In other words, there would be no net environmental benefit from growing wheat for biofuel with such low yields and high N inputs.

There is little data on relative performance of wheat and triticale in 2nd and 3rd cereal positions. Although Overthrow and Carver showed that triticale gave better gross margins due to the lower growing costs, relative yields of triticale and wheat in a severe take-all situation are not available in the published literature. It should be noted that in many experiments (e.g. RL trials) appearance of take-all increases variability in plot yields, and therefore often such trials are abandoned. Therefore larger plots may need to be taken into account when designing rotational experiments designed to explore take-all effects.

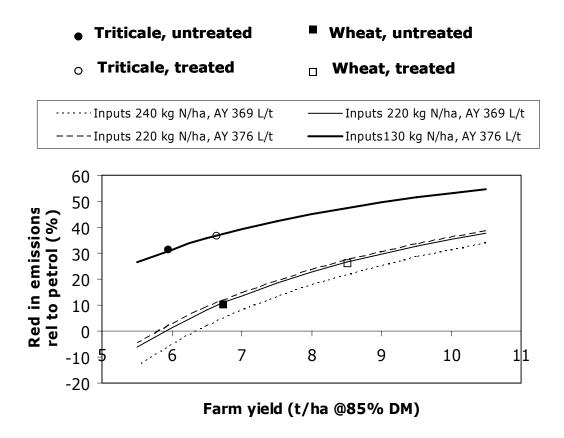
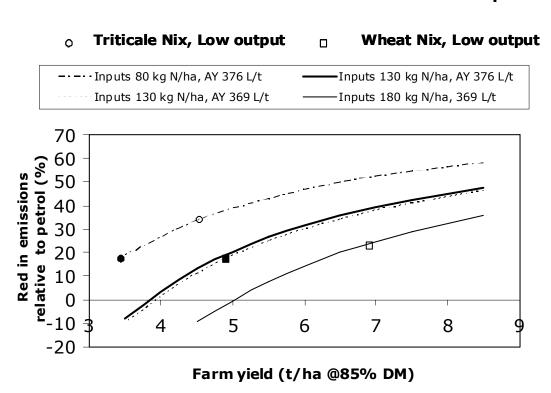


Fig 3. Reductions in emissions per tonne bioethanol produced relative to petrol, for wheat and triticale either treated or untreated with fungicide



Triticale Gleadthorpe Wheat Gleadthorpe

Fig 4. Reductions in emissions per tonne bioethanol produced relative to petrol, for wheat and triticale in low yield potential situations

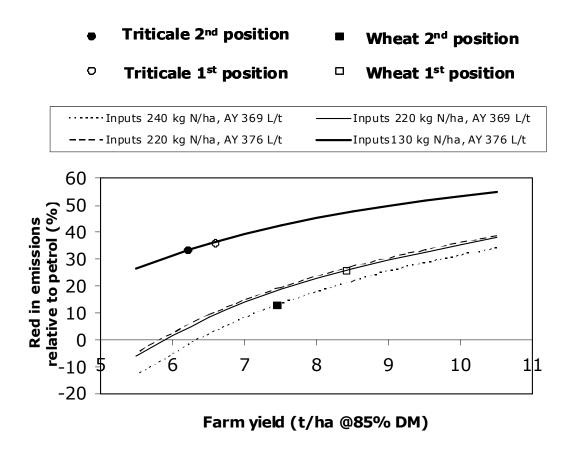


Fig 5. Reductions in emissions per tonne bioethanol produced relative to petrol, for wheat and triticale at different places in the rotation

6.3 Conclusions

- Triticale has been shown to be a feedstock with high potential for bioethanol production, giving alcohol yields comparable with a good distilling wheat (Istabraq) at equivalent grain protein content.
- 2. In all scenarios studied using the HGCA Biofuels calculator, the net benefits in terms of reducing GHG emissions associated with bioethanol production, were greater for triticale than for wheat, principally due the lower N requirement of triticale.
- 3. Some varieties showed better than expected alcohol yields (L/t) based on their starch and protein contents and further work is needed to understand variation

in starch and fermentable sugars in modern triticale varieties, and in different agronomic situations.

- 4. The Scotch whisky lab method for spirit yield was used here to ensure comparison with existing RL data for wheat, but alcohol yields should also be assessed using industrial enzymes representative of those which would be used in a modern bioethanol plant, particularly in terms of controlling viscosity.
- 5. There is considerable uncertainty regarding the average grain protein content of triticale when fertilized at the economic optimum, and further work needs to be carried out in trials where wheat and triticale are grown alongside one another.
- 6. The major advantage of triticale may be in 2nd/3rd cereal positions in the rotation when the yield of wheat tends to be reduced by take-all and more work needs to be carried out to compare these two species side by side in replicated trials.

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Inputs	Quantity
<u>Constants</u> P2O5 K2O Lime	94 kg/ha 55 kg/ha 0 kg/ha
Seed	185 kg/ha
Diesel	141 L/ha
N2O emissions	Proportional to N applied
Grain drying/storage energy	Zero (grain moisture fixed at 15%)
costs Grain transport to distillery	50 km
Process option	NG boiler + steam turbine (heat req. 9.75 GJ/t ethanol; electricity required 1.45 GJ/t ethanol)
Energy balance	14.4 GJ natural gas, 0kWh imported electricity and 396 kWh surplus electricity surplus per tonne ethanol
DDGS used for animal feed	0.31 t/t grain supplied to plant
Ethanol transported by road to end user	50 km
<u>Variables</u> Grain (farm) yield	t/ha @85% DM. Where comparisons have been derived from field experiments, farm yields were estimated from plot yield x 0.8 See tables/scenarios below for further details
Pesticides	0 or 2 kg/ha for untreated/treated respectively
Fertiliser N	80 to 240 kg N/ha
Alcohol yield (FW basis@85%DM)	Wheat: 369 L/t [= 434 L/t DM basis @ 11.5% protein*] Triticale: 376 L/t [= 442 L/t DM basis @10.5% protein*]
	*Based on predictive equation AY (L/t DM basis) = -7.31 x protein +519 (from Smith <i>et al.</i> , 2006)

Annex A Common inputs to Biofuels Calculator model for triticale and wheat

Annex B Inputs and outputs for fungicide effects

Species/ treatment	Variety	Source	Plot yield (t/ha)	Farm yield* (t/ha)	N inputs (kgN/ha)
Wheat treated	Tatabuaa		(10 5)	0.4	220
Wheat, treated	Istabraq	RL 2008/09	(10.5)	8.4	220
Wheat, untreated	Istabraq	RL 2008/09	(8.4)	6.7	220
<u>Triticale</u>					
Triticale, treated	Fidelio	ADAS Rosemaund 2000	(8.2)	6.5	130
Triticale, untreated	Fidelio	ADAS Rosemaund 2000	(7.3)	5.9	130

N inputs and grain yields for different fungicide treatment

* Farm yields used in biofuels calculator

Biofuels calculator model outputs illustrated in Fig. 3.

Species/ treatment	Kg CO₂ eq./t bioethanol produced	% reduction in emissions relative to petrol
Wheat, treated	1707	25.8
Wheat, untreated	2052	10.7
Triticale, treated	1482	35.6
Triticale, untreated	1594	30.7

Annex C Inputs and outputs at low yield potential sites

Species/ treatment	Variety	Source	Plot yield (t/ha)	Farm yield* (t/ha)	N inputs (kgN/ha)
<u>Wheat</u>					
Wheat 1	-	Nix 2007, low yield potential	-	6.8	180
Wheat 2	Beaver/Soissons population mean	ADAS Gleadthorpe 2002/02 Foulkes <i>et</i> <i>al.</i> , 2007	(6.0)	4.8	130
Triticale					
Triticale 1	-	Nix 2007, low yield potential	-	4.4	80
Triticale 2	Average for RL trial varieties	ADAS Gleadthorpe 1999	(4.3)	3.4	80

N inputs and grain yields for 1st cereal, low yield potential scenarios

* Farm yields used in biofuels calculator

Biofuels calculator model outputs illustrated in Fig. 4.

Species/ treatment	Kg CO₂ eq./t bioethanol produced	% reduction in emissions relative to petrol
Wheat 1	1740	22.8
Wheat 2	1922	16.4
Triticale 1	1546	32.8
Triticale 2	1891	17.8

Annex D Inputs and outputs in a second cereal position

Species/ treatment	Variety	Source	Plot yield (t/ha)	Farm yield* (t/ha)	N inputs (kgN/ha)
<u>Wheat</u> 1 st Wheat 2 nd Wheat	Istabraq Istabraq	RL 2008/09 Estimated from 1 st wheat yield with 12% yield reduction (-1 t/ha)	(10.5)	8.4 7.4	220 240
<u>Triticale</u> 1 st Triticale 2 nd Triticale	Grenado Grenado	RL 2009/09 Estimated from 1 st triticale yield with 6% yield reduction	(8.2)	6.6 6.2	130 130

N inputs and grain yields for 1st and 2nd cereals, with moderate take-all severity

* Farm yields used in biofuels calculator

Biofuels calculator model outputs illustrated in Fig. 5.

Species/ treatment	Kg CO₂ eq./t bioethanol produced	% reduction in emissions relative to petrol
1 st Wheat	1707	25.8
2 nd Wheat	2012	12.5
1 st Triticale	1470	36.1
2 nd Triticale	1544	32.9