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MANAGEMENT OF BREADMAKING WHEAT: EFFECTS OF EXTRA NITROGEN ON YIELD, GRAIN AND FLOUR QUALITY

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by

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* The Campden and Chorleywood Food Research Association (CCFRA) was formed in January 1995 by merger of the Campden Food and Drink Research Association (CFDRA) and the Flour Milling and Baking Research Association (FMBRA).

CONTENTS

1. ABSTRACT

Between 1988-1991, a total of 45 field trials were carried out by ADAS on breadmaking varieties of wheat testing the effect of extra N applied in addition to the normal N rate applied for yield. In Trial Series A, the effect of different rates of extra N (0-180 kg/ha) was tested applied either as ammonium nitrate prills at GS32 (2nd node stage) or as a foliar urea spray at GS75 (milky ripe stage). In Trial Series B, the effect of different timings of application of a single rate of extra N as foliar urea (30 or 40 kg/ha) was tested. The effect of sulphur fertiliser was tested at all sites.

Measurements of grain yield, grain quality and nitrogen recovery in grain were taken at all sites. At selected sites, full milling and baking tests were carried out by the Flour Milling and Baking Research Association (FMBRA). At a few sites, detailed protein biochemistry and protein fractionation studies were also carried out.

Both forms of extra N gave substantial increases in grain protein, but foliar urea consistently gave larger increases than ammonium nitrate. Protein responses were greater where the base level of protein was low - where base protein content was 10.5-12.0%, application of 30 kg/ha extra N as foliar urea increased grain protein by 0.67%, and by 0.46% when applied as ammonium nitrate prills. Timing of foliar urea application was found to be important with the optimum time being between GS70-79 (milk development). Earlier or later application was generally less effective.

At most sites there was a grain protein response up to high rates of extra N, with increases of over 3% grain protein commonly achieved. Recovery of extra N in the grain was low and averaged about 30% from a 30 kg/ha N application. At higher rates, N recovery dropped rapidly. Recovery was no worse from extra N applied as foliar urea than ammonium nitrate, and at several sites was significantly better.

Baking tests showed that both forms of extra N produced protein that was useful for breadmaking using a Long Fermentation Process (LFP). Some limited comparisons at high protein sites of the LFP baking method with the Chorleywood Bread Process (CBP) showed

much reduced responses of CBP loaf volume to protein than for LFP loaf volume. There were few effects on milling parameters, though foliar urea consistently increased flour colour grade.

Protein biochemistry studies confirmed that the proteins produced by both forms of extra N occurred as high molecular weight gliadins and glutenins. There were indications that extra N applied as late foliar urea produced better quality proteins than when applied as ammonium nitrate.

Application of sulphur fertiliser had inconsistent and erratic effects, though the trial sites were not selected to be in sulphur deficient situations. The sulphur treatments used had little effect on the concentration of sulphur in grain.

2. INTRODUCTION AND OBJECTIVES

2.1 Introduction

The UK flour milling industry requires nearly 4 million tonnes of breadmaking quality wheat each year (NABIM, 1993), and when harvest conditions are favourable, nearly 90% of this wheat can be home-grown. However, since current varieties suitable for breadmaking generally have a lower yield potential than those for feed markets, it follows that it will only be profitable to grow breadmaking wheat varieties if a quality premium continues to be paid above feed varieties and if farmers consistently achieve this premium by producing grain to the required quality from a high proportion of their crops.

Reforms under the Common Agricultural Policy (CAP) and the GATT agreement, mean that maximum profits from wheat production will only be achieved if the required grain quality is reliably achieved by farmers in response to the demands of the market, whether it be a home or export market.

In addition to other grain quality parameters such as moisture, Hagberg Falling Number and specific weight, grain protein is of critical importance. Additional nitrogen above that required for yield alone, is commonly applied by farmers to increase grain protein content in the hope of meeting the required specifications for breadmaking wheat, and of attracting a financial premium for quality.

In practice, this extra nitrogen is applied as solid ammonium nitrate or as a foliar urea spray during grain filling. Data from the Survey of Fertiliser Practice (MAFF, 1990) shows that 42% of the cropped area of breadmaking wheats receives extra nitrogen with the specific intention of raising the grain protein content. The average application was 41 kg/ha N; 70 % of this N was applied as solid prilled or granular N, and 30% as a late foliar urea spray.

Due to both economic and environmental factors, it is important for all farmers to adopt the most effective approach to nitrogen use when growing breadmaking wheats - or indeed any other crop. Concerns about the use of excessive amounts of nitrogen fertiliser and the

associated increased risk of pollution of watercourses mean that any use of nitrogen must be fully justified and carried out in an efficient way, and only when needed.

Earlier work suggested that a foliar urea spray applied at GS75 (milky-ripe stage) produced increases in protein content but did not appear to be totally effective in improving breadmaking quality (Hook et al, 1989). Against a background of rather poor harvesting conditions in this work, it proved impossible to reach a definite conclusion on this key point.

Further, the standard method used by the milling industry to measure 'protein' content is to analyse grain for total nitrogen (N) and multiply by a factor to obtain a protein content at 86% dry matter. This method of analysis does not distinguish between different forms or qualities of protein, or nitrogen in non-protein forms. Protein quality is very important for breadmaking performance and there have been concerns that late applications of nitrogen fertiliser may not be fully converted by the plant into the useful forms of protein that are essential for breadmaking.

For breadmaking wheat varieties, a positive correlation between protein content and loaf quality should exist for the normal range of protein content: the slope of this relationship depends upon the variety concerned and the breadmaking process used (Kent, 1982). Protein is not evenly distributed throughout the wheat grain. Concentration increases from the centre of the grain outwards, and the protein associated with the aleurone layer and outer layers of the grain has no function in breadmaking (Kent, 1982).

It is vital to the end-user, the miller and ultimately the baker, to establish whether the additional protein produced by nitrogen treatment is functional in breadmaking terms. Equally, the farmer needs to be assured that any fertiliser applications are carried out in the most economically advantageous and effective manner. An understanding of the types and quantities of the different types of storage proteins laid down in the endosperm is crucial.

Wheat intake tests, often used in agronomy experiments to measure quality, only indicate whether a wheat is likely to be accepted at mill intake and provide a guide to breadmaking potential. In this work, breadmaking assessments were used, backed up by biochemical

analysis of changes in wheat protein composition, to establish the effects of agronomic practices on end-use quality. The combination of agronomy expertise and full quality assessment of scientifically controlled experimentation aims to address these issues crossing the food chain from farmer to baker.

However, it is not just the nitrogen supply which is important in terms of the types and quantities of storage proteins laid down in the wheat grain. Sulphur is an essential and important nutrient and it is well recognised that atmospheric levels of sulphur are reducing rapidly resulting in the increased possibility of deficiency in crops (McGrath et al, 1993). It is not only potentially important to maximise grain yield, but it is also vital in order to maximise the functionality and quality of protein. Inadequate concentrations of sulphur will reduce the amount of essential cross-linkages present within wheat proteins.

The need for sulphur application to improve grain yield, protein quantity and quality is not well established in Britain, particularly when in combination with late applications of nitrogen. In this study, some sulphur treatments were included, but the trial sites were not necessarily located in areas or on soil types likely to be sulphur deficient.

2.2 Objectives

The objectives of the project were as follows:

i) To establish if grain N increases, produced by foliar urea-N spray applications, also improve the milling and breadmaking quality of wheat.

ii) To establish the effect of (a) different rates and (b) different timings of foliar urea-N, with or without additional sulphur, on grain N and milling and breadmaking quality.

iii) To develop improved recommendations for nitrogen use on breadmaking wheat varieties in order to produce grain of high quality and to avoid ineffective nitrogen applications that will increase the risk of nitrate pollution.

iv) To gain some insight into the biochemical consequences of differing nitrogen treatments in terms of protein quality and composition.

2.3 Interim Reports

Various interim publications and presentations have been made during the lifetime of the project including scientific conferences, popular articles and farm meetings.

Notable items include Dampney et al (1990), Salmon et al (1990), Pritchard (1993), the HGCA R&D conference Jan 1992, Cereals 94, various FMBRA conferences and the MAFF/HGCA roadshows in autumn 1994 (6 conferences).

3. TRIALS DESIGN AND METHODOLOGY

3.1 Trials design and locations.

There were 2 separate, fully replicated, experimental designs within the Project:

Trial Series A studied the effect of different rates of application of extra N applied either as ammonium nitrate prills at GS32 (2nd node stage) or as foliar urea at GS75 (milky ripe stage. Trial Series B studied the effects of different timings of application of foliar urea.

Trials were carried out over 4 years (harvests 1998-1991). In each year, there were 6 separate trial sites for each trial series, located in England on commercial farms or ADAS Research Centres. Over the 4 years of the work, a total of 22 trials in Series A and 23 trials in Series B were successfully completed.

The design and treatments for each series of trials are outlined in Sections 3.1.1 and 3.1.2. Trial locations and site details are given in Tables 1 and 2. All trials were carried out on a recommended breadmaking wheat variety, mostly Mercia. All treatments were applied by hand and trials harvested using a small plot harvester.

Although a range of nitrogen rates was used in these trials, these were applied as extra N in addition to a base dressing of N applied for yield at conventional spring timings (GS23 and GS31). Estimation of the optimum rate of total N for each site was not possible and was not an objective of the project. The base rate of N used at each trial site was judged to produce grain with a protein content of 10.0-10.5%, and at several sites was different, commonly slightly lower, than normal recommendations.

This trial design was necessary so that the use of different rates of extra N would produce a range of grain samples with protein contents in the critical range of 10.0-12.0% protein where differences in breadmaking quality might be expected.

The following assessments were carried out by ADAS at most sites:

- grain yield
- grain moisture
- grain nitrogen (by NIR)
- grain SDS sedimentation volume
- grain Hagberg Falling Number
- grain specific weight
- leaf scorch
- crop lodging

At selected sites, full milling and baking tests were carried out by FMBRA (see section 6). Protein biochemistry tests were carried out at a few sites (see section 7).

3.1.1 Trial design and location. Series A. Rate of foliar urea.

All plots received 40 kg/ha at GS 23 plus the balance of the basal N dressing at GS 31. All nitrogen (including GS 23 and GS 31) was applied by hand. The total base N rate (GS 23 + 31) was judged to produce grain at about 10.5% protein for the site. This was judged on the basis of previous farm experience and site N fertility, sometimes being less than the amount of nitrogen that would normally be recommended for the crop.

Treatment	Ammonium nitrate (kg/ha N) GS23	GS31	GS32	Foliar urea (kg/ha N) GS75	GS75 -4 days	GS75 -2 days	GS75 +2 days
1	40	*	0	0	0	0	0
2	40	*	0	0	0	30	0
3	40	*	0	0	30	30	0
4	40	*	0	0	30	30	30
5	40	*	0	30	30	30	30
6	40	*	0	30	60	60	30
7	40	*	30	)			
8	40	*	60	)			
9	40	*	90	) Nil foliar urea			
10	40	*	120	)			
11	40	*	180	)			
12	40	*	0	0	0	30 + S	0
13	40	*	0	0	30 + S	30	0
14	40	*	0	30 + S	30	30	30

* The N rate at GS31 varied between sites according to site conditions.

Design: Small plots (40-100 m^2) with 4 replicates of each treatment in randomised blocks = 56 plots.

Foliar urea: 30 kg/ha N in 300 litres/ha. For the 60 kg/ha rate, two applications of 30 kg/ha were applied during the same day but allowed to dry between applications.

Sulphur: 10 kg/ha S as Thiovit was applied at GS 75. In addition, in 1990 and 1991, an extra 50 kg/ha S as gypsum was applied in early spring.

3.1.2 Trial design and locations. Series B. Timing of foliar urea.

All plots received 40 kg/ha N at GS23 plus the balance of the base N dressing at GS 31. All nitrogen (including GS 23 and GS 31) was applied by hand. The total base N dressing (GS23 + GS31) was judged to produce grain at about 10.5% protein. This was on the basis of previous farm experience and site N fertility, sometimes being less than the amount of nitrogen that would normally be recommended for the crop.

Foliar urea treatments (kg/ha N).

1. Nil
2. Nil
3. 40 kg/ha urea - N when flag leaf fully emerged (early June)
4. " + 10 days
5. " + 20 days
6. " + 30 days
7. " + 40 days
8. " + 50 days
9. " + 60 days
10. " + 70 days (mid August)
11. Treatment 6 plus sulphur
12. Treatment 7 plus sulphur
13. Treatment 8 plus sulphur

Design: Small plots (40-100 m^2) with 4 replicates of each treatment in randomised block = 52 plots.

Foliar urea: 40 kg/ha urea N was applied in 300 litres/ha (30 kg/ha at sites 27, 28, 29, 30).

Sulphur: 10 kg/ha S as Thiovit was applied at GS 75. In addition, in 1990 and 1991, an extra 50 kg/ha S as gypsum was applied in early spring.

Table 1. Trial locations and site details (Series A. Rate of foliar urea)

Site Location	Soil type*	Variety	Base N (kg/ha)	N Index
1988 harvest.				
1. Glympton, Oxon	CL over limestone	Mercia	180	1
2. Bapton, Wilts	Shallow ZyCL over chalk	Mercia	150	0
3. Kneesall, Notts	ZyCL over Keuper Marl	Mercia	150	1
4. Longhirst, N'land	SL over boulder clay	Mercia	160	0
5. Nocton, Lincs	SCL over limestone	Avalon	149	1
6. Mears Ashby, Northants	CL over boulder clay	Mercia	153	1
1989 harvest.				
7. Syerscote, Staffs	CL over Keuper Marl	Mercia	150	0
8. Hargrave, Suffolk	CL over boulder clay	Mercia	165	1
9. Boyt, Dorset	Shallow ZyCL over chalk	Mercia	150	0
10. Folkingham, Lincs	CL over boulder clay	Mercia	170	0
11. Bladon, Oxon	SL over limestone	Mercia	180	0
12. Bamburgh, N'land	SZL over sst/shale	Mercia	220	1
1990 harvest.				
13. ADAS Bridgets	ZyL over chalk	Avalon	225	0
14. Kempston, Beds	CL over boulder clay	Mercia	180	0
15. Kidlington, Oxon	SCL over limestone	Mercia	180	0
16. Frampton, Lincs	ZyL over alluvium	Mercia	50	1
17. ADAS H.Mowth.	SCL over chalk	Mercia	180	1
18. Roundway, Glos	Shallow ZyCl over chalk	Mercia	150	0
1991 harvest.				
19. Swaton, Lincs	SCL over boulder clay	Mercia	190	1
20. B.Canning, Wilts	Shallow ZL over chalk	Mercia	150	0
21. ADAS Bridget's	ZL over chalk	Mercia	200	1
22. Bladon, Oxon	SCL over limestone	Mercia	c.180	0
23. ADAS H.Mowth.	SCL over chalk	Mercia	180	1
24. Milton Ernest, Beds	CL over boulder clay	Mercia	152	1

* Topsoil texture and geological parent material

Results are discussed of 22 sites. Data from the following sites are suspect and not included in the Results section.

Longhirst, 1988 - complete, overall lodging caused variable data.
High Mowthorpe, 1991 - missing data from 13 plots.

Table 2. Trial locations and site details (Series B. Timing of foliar urea)

Location	Soil type*	Variety	Base N (kg/ha)	N Index
1988 harvest.				
25. Maidenwell, Kent	ZyCL over chalk	Avalon	150	1
26. Brampton, Cambs	CL over boulder clay	Avalon	139	1
27. ADAS Terrington,	ZL over marine alluvium	Mercia	110	1
28. ADAS Rosemaund,	SCL over old red sst	Mercia	126	1
29. ADAS Boxworth,	CL over boulder clay	Mercia	225	0
30. Detling, Kent	ZyCL over chalk	Avalon	150	1
1989 harvest.				
31. ADAS Rosemaund,	ZL over old red sst.	Mercia	130	1
32. NAS Morley	SCL over boulder clay	Avalon	144	1
33. ADAS Terrington,	ZyCL over silt	Mercia	240	1
34. Bethesden, Kent	CL over weald clay	Avalon	200	0
35. ADAS Boxworth	CL over boulder clay	Mercia	120	1
36. Maidenwell, Lincs	ZyCL over chalk	Mercia	150	1
1990 harvest.				
37. ADAS Rosemaund,	ZyL over old red sst.	Mercia	125	1
38. ADAS Terrington,	SZL over marine alluvium	Pastiche	220	0
39. ADAS Boxworth	CL over boulder clay	Mercia	130	1
40. Bethesden, Kent	CL over weald clay	Mercia	200	0
41. Sutterby, Lincs	ZyCL over chalk	Mercia	140	1
1991 harvest.				
42. ADAS Rosemaund,	ZyL over old red sst.	Mercia	136	1
43. ADAS Terrington,	SZL over marine alluvium	Mercia	200	0
44. ADAS Boxworth	CL over boulder clay	Mercia	120	1
45. Bethesden, Kent	ZyCL over weald clay	Mercia	200	0
46. Rigsby, Lincs	ZyCL over chalk	Mercia	150	1
47. Milton Ernest, Beds	CL over boulder clay	Mercia	152	1

* Topsoil texture and geological parent material

3.2 Methods of laboratory analysis

3.2.1 Grain quality

Grain quality analyses were carried out in ADAS laboratories using standard analytical techniques (MAFF, 1986). Grain nitrogen content was determined by NIR (Near Infra Red).

3.2.2 Milling and baking quality.

Grain samples from selected trial sites were analysed by FMBRA for milling and breadmaking quality (see Table 3). All selected sites had a satisfactory Hagberg Falling Number value for breadmaking and with a range of grain protein contents. Where possible, sites with extremely high or low protein contents, or with a limited range in values, were excluded from the assessment of milling and baking quality.

Milling and breadmaking quality assessments were carried out according to standard procedures. All samples of wheat were received at FMBRA with a moisture content less than 15%. Samples were cleaned using a Carter-Day Dockage tester and conditioned to 16.0% moisture by adding water and allowing to stand for between 16 and 23 hours. Wheat samples were milled using a standard comparative milling process to produce a white flour containing the minimum quantity of bran contamination. The amount of white flour produced from a fixed weight of wheat is quoted as the percentage flour extraction.

Measurements of flour quality were determined according to standard FMBRA procedures (Anon, 1991). Full details are given in previous interim reports. In particular, the following measurements were used to assess final flour quality in this project.

Flour colour grade

Flour colour grade, which provides a measurement of bran contamination in a white flour sample by the reflectance of a flour/water paste, was determined using the Kent-Jones and Amos Colour Grader.

Flour moisture content

Moisture content was measured by two procedures, namely oven and NIR according to standard procedures.

Table 3. Trial sites selected for milling and breadmaking quality assessments at FMBRA.

Harvest year	Trial series	Site (No)	Variety	Control treatment	
				Mean grain protein content	Mean grain Falling Number
1988	A	Kneesall(3)	Mercia	9.20	339
	B	Detling(30)	Avalon	10.63	217
1989	A	Boyt(9)	Mercia	10.53	310
	A	Folkingham(10)	Mercia	9.87	371
	A	Hargrave(8)	Mercia	9.16	313
	B	Bethesden(34)	Avalon	11.75	351
	B	Morley(32)	Avalon	10.96	378
	B	Terrington(33)	Mercia	11.24	298
1990	A	Frampton(16)	Mercia	8.89	337
	A	Roundway(18)	Mercia	9.56	372
	B	Bethesden(40)	Mercia	11.58	356
	B	Sutterby(41)	Mercia	10.83	343
	B	Terrington(38)	Pastiche	11.95	399
1991	A	Bishops Canning(20)	Mercia	11.88	302
	A	Milton Ernest(24)	Mercia	12.32	350
	B	Boxworth(44)	Mercia	10.81	332
	B	Milton Ernest(47)	Mercia	12.24	347

Flour protein content

Protein content was determined in two ways: by chemical analysis of the nitrogen content of the sample using a standard Kjeldahl procedure and using NIR, calibrated against Kjeldahl protein values.

Breadmaking assesment

A bulk Long Fermentation Process (LFP) was used for breadmaking throughout this study, but in the final year of the work this was supplemented with a standard, high-speed mixing Chorleywood Bread Process (CBP) baking test on samples from Trial Series A where sample size allowed.

In April 1990, potassium bromate was removed from the list of permitted bread improvers. This slow-acting oxidising agent had proved very effective in bulk fermentation baking processes, and it's removal led to a further decline in the use of such traditional breadmaking methods. Modifications to the recipe for the CBP process permitted the production of high quality commercial bread when ascorbic acid only was used (Collins, 1989). For comparative purposes in this Project, LFP test baking procedures included potassium bromate as an improver throughout the work.

The CBP test includes the addition of a fungal alpha-amylase supplement designed to bring all flour samples to the common alpha-amylase level of 40 Farrand units. This equalisation of amylase levels is not carried out in the standard bulk fermentation procedure, but the procedure includes an appropriate level of sugar to ensure that a substrate for fermentation is not lacking.

Gluten

Analyses for the quantity of wet gluten obtained from flour samples under a standard gluten washing test (Anon, 1991) were also carried out on a maximum of two replicates from both Trial Series but in the final year of the project only. Gluten quality was assessed by the Gluten Index method (Perten, 1990).

Procedure for Long Fermentation Process (LFP)

Bread type:	400g, white	
Mixing machine:	McDuffy	
Control recipe:		
	% of flour weight	g/mix
Flour	100	280
Yeast (compressed)	2.0	5.6
Salt	2.0	5.6
Water	As determined by Farinograph using 600 line or Simon Extrusion Gun technique.	
Sugar	2.0	5.6
Potassium bromate	0.0015	0.42
Dough processing:		
Mixing machine:	McDuffy pin mixer	
Batter speed:	Slow speed, 2 min	
Dough temperature:	27 +/- 1°C	
First moulding:	Mono 6 ins bench moulder (R7mm, P41mm)	
First proof:	2 hours at 27°C	
Final moulding:	Mono bench moulder (R7mm, P41mm)	
Pan size:	160mm x 98mm, 83 mm deep	
Shape:	Unlidded	
Proving conditions:	32°C, humidity to prevent skinning	
Proving time:	50 minutes	
Baking temperature:	232°C	
Oven type:	Simon electric reel	
Baking time:	32 minutes	
Baking humidity:	Water for steam	
Cooling:	Open rack at ambient	
Storage:	Cupboard ambient	

Procedure for Chorleywood Bread Process (CBP)

Bread type: 400g, white
Mixing machine: Morton

Control recipe:

	% of flour weight	g/mix
Flour	100	1400
Yeast (compressed)	2.5	35
Salt	2.0	28
Water	As determined by Farinograph using 600 line	
Fat (Ambrex, slip point c.45°C)	1.0	14
Ascorbic acid (100ppm AA)	0.01	0.14

The alpha-amylase activity of the flour is adjusted to 40 FU by the addition of fungal alpha-amylase. Flour 'base' level of alpha-amylase is estimated from the Falling number.

Dough processing:

Mixing machine:	Variable speed Morton
Beater speed:	300 rev/minute
Work input:	11 Wh/kg
Pressure:	Atmospheric
Dough temperature:	30.0 +/- 1°C
Scaling:	By hand to 454g
First moulding:	Mono 5 ins bench moulder (R8mm, P43mm)
First proof:	10 minutes at ambient temperature
Final moulding:	Sorenson commercial (R7, W5.5, P1.25)
Pan size:	160mm x 98mm, 83mm deep
Shape:	Unlidded
Proving conditions:	43°C, humidity to prevent skinning
Proving height:	10 cm (max. time 60 minutes)
Baking temperature:	260°C
Oven type:	Direct gas-fired Reel (6 tray)
Baking time:	25 minutes
Baking humidity:	No steam injected
Cooling:	Open rack at room temperature
Storage:	Closed cupboard overnight at 21°C

3.2.3 Protein biochemistry

The protein biochemistry of flours from selected treatments at selected sites was examined - see Table 4. Because of the complex and costly nature of these determinations, the nature and extent of these tests varied from year to year (Table 5).

Table 4. Sites selected for protein biochemistry studies.

Harvest year	Trial series	Site (No)	Variety
1988	A	Kneesall (3)	Mercia
1989	A	Boyt (9)	Mercia
	A	Hargrave (8)	Mercia
	B	Morley (32)	Avalon
	B	Terrington (33)	Mercia
1990	A	Frampton (16)	Mercia
1991	A	Bishops Canning (20)	Mercia

Table 5. Summary of biochemical studies applied in the different harvest years.

1988 (All treatments)	Protein content from total aminoacid analysis Sulphur aminoacid residues (cysteine and methionine) Acid-soluble free aminoacids Omega-gliadin content (sulphur-poor protein)
1989 (Selected plots)	Total protein (Kjeldahl assay) Protein in (albumin + globulin), gliadin, glutenin subfractions Cysteine residues in the above protein fractions
1990 (All treatments)	As for 1989 above, except Lowry protein assay, gel protein weight and elastic modulus.
1991 (All treatments)	As for 1990 above

Aminoacids recovered after acid hydrolysis; calculation of protein content

Single flour samples from each plot were hydrolysed by 6M hydrochloric acid at 105°C for 23 hours. The resultant aminoacids were extracted from the residue after evaporation under vacuum, and analysed on the LKB-alpha aminoacid analyser by ion-exchange chromatography and detection with ninhydrin. Recovery during work-up was estimated with an internal standard of the non-protein aminoacid norleucine, and the instrument was calibrated with a standard solution of aminoacids including norleucine. The results were used to calculate the aminoacid-derived N content of the flours, and the factor 5.7 was then used to convert the results to an apparent protein content.

Sulphur aminoacids after performic acid oxidation

The sulphur-containing aminoacids cysteine and methionine cannot be estimated reliably by the above procedure because oxidation of the sulphur atoms to varying extents gives a mixture of many derived forms. Hence the flours were pre-treated with performic acid, essentially as described by Timms et al. (1981), such that these aminoacids could be estimated as single highly oxidised forms, cysteic acid and methionine sulphone, in the above acid hydrolysis procedure.

Cysteine after oxidation with dimethyl sulphoxide

Cysteine residues in the wheat proteins were measured by a labour-saving protocol developed from the methods of Spencer and Wold (1969) and Williams et al. (1979). Oxidation to cysteic acid was achieved by adding 2% dimethyl sulphoxide to the standard acid hydrolysis system, and evaporation to dryness was replaced by careful adjustment of the hydrolysis mixture to pH 2.2 with sodium hydroxide. Cysteic acid and the norleucine standard were then measured in the hydrolysis mixture directly.

Acid-soluble "free" aminoacids

Aminoacids in low molecular weight non-protein forms were extracted from flours with a solution of the protein-precipitating reagent sulphosalicylic acid, essentially as described by Timms et al. (1981). Portions of the extracts were heated for 1 hour at 100°C to hydrolyse asparagine and glutamine to aspartic and glutamic acid. Aminoacid analysis was conducted as above on parallel samples before and after heating at 100°C, to enable estimation of both the amides and the free acids, since they were not properly resolved in the chromatographic system being used, but proved to be major components of the extracts. The results were calculated as free aminoacid-N, and expressed as percentages of the total Kjeldahl N.

Omega-Gliadin content (sulphur-poor gluten protein)

The relative omega-gliadin contents of a set of flours (all of the same variety and therefore with the same gliadin fingerprint) were estimated from the electophoretic patterns of stained protein bands, produced by polyacrylamide gel electrophoresis at pH 3.1 (Salmon and Burbridge, 1985). The patterns were scanned with a Chromoscan 3 integrating densitometer, and the height of the most prominent peak in the envelope of omega-gliadins was taken as representative of the amount of omega-gliadin.

Extraction of sub-fractions of flour proteins

An estimate of the amount of protein and cysteine residues in the sub-fractions was obtained using a modified and simplified version of the traditional Osborne sequential extraction procedure to separate operational fractions of the flour protein. These fractions, being operational, were similar but probably not identical to other partial protein extracts that have been given the same names in other studies of flour proteins.

Flour (750mg) was extracted with three solvents (7.5 ml) in turn, each one with gentle shaking to suspend the flour particles at 25°C for 1 hr:

-- 0.5M NaCl to extract albumins + globulins

-- 60% v/v propan-2-ol to extract gliadins
-- 1.5% w/v sodium dodecyl sulphate (SDS) solution with 5mM dithiothreitol (DTT) to extract glutenins

After each period, the mixture was centrifuged gently (300 x g, 10 min, to avoid gluten separation after the first solvent), and the extract removed from the pelleted residue by decantation.

A second portion of flour was extracted directly in the third solvent (SDS + DTT) to obtain an additional measure of the total protein and cysteine present. Aliquots of the extracts were diluted into 1% SDS solution for protein estimation by the Lowry colorimetric procedure (see below), or into acetone (5 volumes) for precipitation of protein prior to estimation of cysteine residues as cysteic acid (see above method). In some cases, such precipitates were also used to estimate protein content via the Kjeldahl-N procedure, as a check on the Lowry method.

Estimation of protein by the Lowry/Folin method

As a consequence of the large number of flour extracts that had to be examined, it was necessary to devise a modified and semi-automated version of the traditional Lowry procedure with copper tartrate and Folin phenol reagent. The modification to allow the use of SDS solution, in order to keep the gliadin and glutenin fractions in solution, was based on that of Markwell et al. (1978), with bovine serum albumin (BSA) as the standard protein. Semi-automation was achieved by scaling down the reaction mixtures to fit in the wells of a standard 96-well microlitre plate, along the lines indicated by Fryer et al. (1986) and Stoscheck (1990). All assays and standards were run in triplicate, and the developed colour was read automatically by a Biotek 311 plate reader; the proprietary software then fitted a curve to the standard points and calculated the unknowns in terms of BSA equivalents.

Gel-protein weight - based on the original procedure of Graveland

10g of flour was defatted with 25ml petroleum ether (b.p. 40-60°C) for 1 hour, then filtered and air dried. 5.0g of defatted flour was stirred gently with 90ml of 1.5% sodium

dodecylsulphate(SDS) solution at 10°C for 10 minutes. The flour suspension was centrifuged at 63000 x g for 40 minutes, before decanting off the supernatant SDS solution. The gel layer was scraped off and recorded as grams of gel-protein per 5g of flour.

Gel-protein elastic modulus (G')

The elastic modulus (G') was measured using a small-strain oscillatory rheometer such as the Bohlin VOR. Gel-protein, prepared as above, was loaded into the rheometer after a 30 minute relaxation period at 10oC.

The experimental conditions for the Bohlin were:-

System configuration	concentric cylinder C14
Torsion bar	17.73 g/cm
Frequency	0.1 to 20 Hz
Strain	0.142
Temperature	25°C
Amplitude	69%

Duplicate determinations were carried out on each sample and the mean of the results obtained at 1 Hz is quoted.

4. DATA ANALYSIS

A statistical analysis of variance was carried out on data from each site for main treatments and interactions.

Because of the volume of information, these details are not included in this report. Statistically significant effects for the main crop and grain parameters at each site are given in the Appendices 1-15.

A full set of milling and baking quality data for each trial examined by FMBRA is given in Appendix 16 for Trial Series A and Appendix 17 for Trial Series B.

5. RESULTS - YIELD, GRAIN QUALITY AND NITROGEN RECOVERY

5.1 Trial Series A. Effect of rate of extra N ammonium nitrate (GS 32) or foliar urea (GS 75).

5.1.1 Grain yield and lodging (see Appendix 1 for site data).

The trials reported here were not designed to determine the optimum level of nitrogen for grain yield. However, the relative effects of extra N as ammonium nitrate or foliar urea can be assessed at 22 sites. Significant differences ($p<0.05$) are shown below:

a)	no effect from either form of N	8 sites
b)	equal increase from both forms of N	4 sites
c)	higher yield from ammonium nitrate	4 sites
d)	higher yield from foliar urea	1 site
e)	decrease from ammonium nitrate	0 sites
f)	no increase, decrease from foliar urea	5 sites
g)	increase from sulphur application	3 sites
h)	decrease from sulphur application	1 site

The overall effect of extra N treatments on grain yield is shown in Figure 1. Both forms of extra N applied at 30 kg/ha N increased yield but there was a larger increase from ammonium nitrate (+0.16 t/ha) than from foliar urea (+0.10 t/ha). There was a small additional benefit from higher rates of N as ammonium nitrate but not from foliar urea.

At N rates above 30 kg/ha, use of foliar urea gave a reduced yield benefit, and could not achieve the same maximum yield that was possible through use of ammonium nitrate applied earlier in the season. Previous work (Sylvester-Bradley et al, 1982) has also shown the inefficiency of very late applied N on grain yield. However, a net negative effect on yield (below the control level) from foliar urea was only found at very high rates of urea application above 120 kg/ha N.

This adverse effect was probably due to leaf scorch or nitrogen (ammonium) toxicity effects from the foliar urea. However, although leaf scorch from foliar urea occurred at nearly all

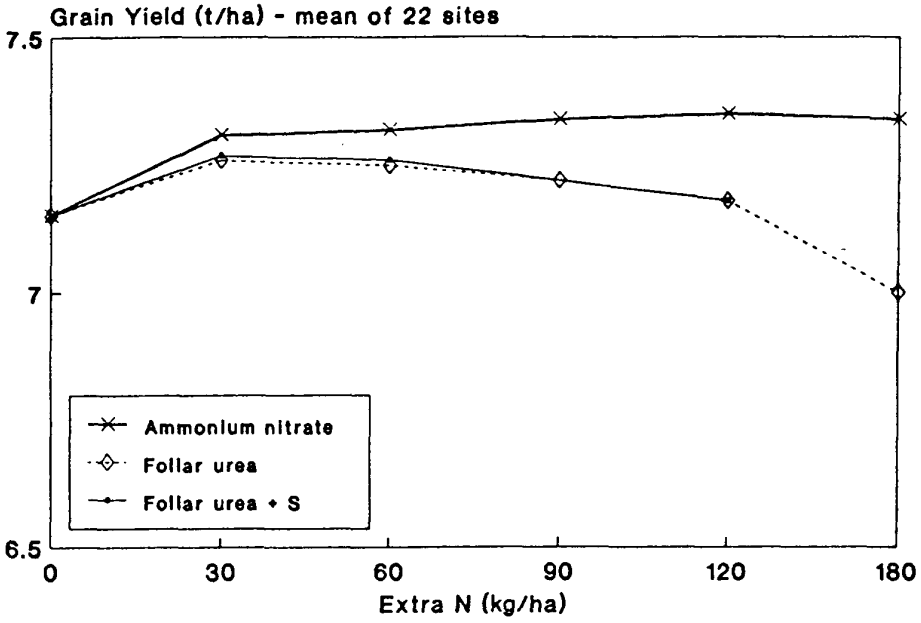


Figure 1. Mean effect of extra N on grain yield (t/ha at 86% DM) - mean of 22 sites.

sites, this could not be clearly associated with the yield differences. At normal commercial rates of application (30-60 kg/ha N), scorch from foliar urea had very little effect of yield.

There were, however, different types of response between sites. There were 9 sites where yield was increased by extra N. At 4 sites there were no differences between the forms of extra N (eg. Figure 2a) and at another 4 sites ammonium nitrate gave a higher yield (eg. Figure 2b). There was only one, low-yielding site (site 11, Bladon 1989) where the average yield was higher from foliar urea.

At some responsive sites (eg. Figure 2b), it was apparent that the maximum yield achieved from extra N as ammonium nitrate could not be achieved through use of foliar urea. This confirms the lower potential of foliar urea for achieving an increase in grain yield.

At most sites, lodging was not a problem. However, at 4 sites significant lodging ($p<0.05$) did occur which was influenced by nitrogen treatment. At all of these sites, lodging was more

severe where extra N was applied as ammonium nitrate. Use of foliar urea had little effect on lodging presumably due to the later timing of this application beyond the time of sensitivity to lodging. The effect of extra N on lodging is shown in Figure 3.

Although modern breadmaking varieties are normally of good lodging resistance, use of extra N as foliar urea to minimise lodging could be an important factor in some situations. However, massive lodging caused by storm conditions is not likely to be influenced by such treatment differences.

There were significant yield increases from sulphur application at 3 sites, all located on shallow soils over chalk or limestone in South or South West England - Glympton 1988 (Oxon), ADAS Bridgets 1990 (Hants), Bishops Canning 1991 (Wilts). Mean responses ranged between 0.13-0.36 t/ha (mean 0.23 t/ha). However, at site 8 (Hargrave 1989) there was a significant yield decrease due to sulphur.

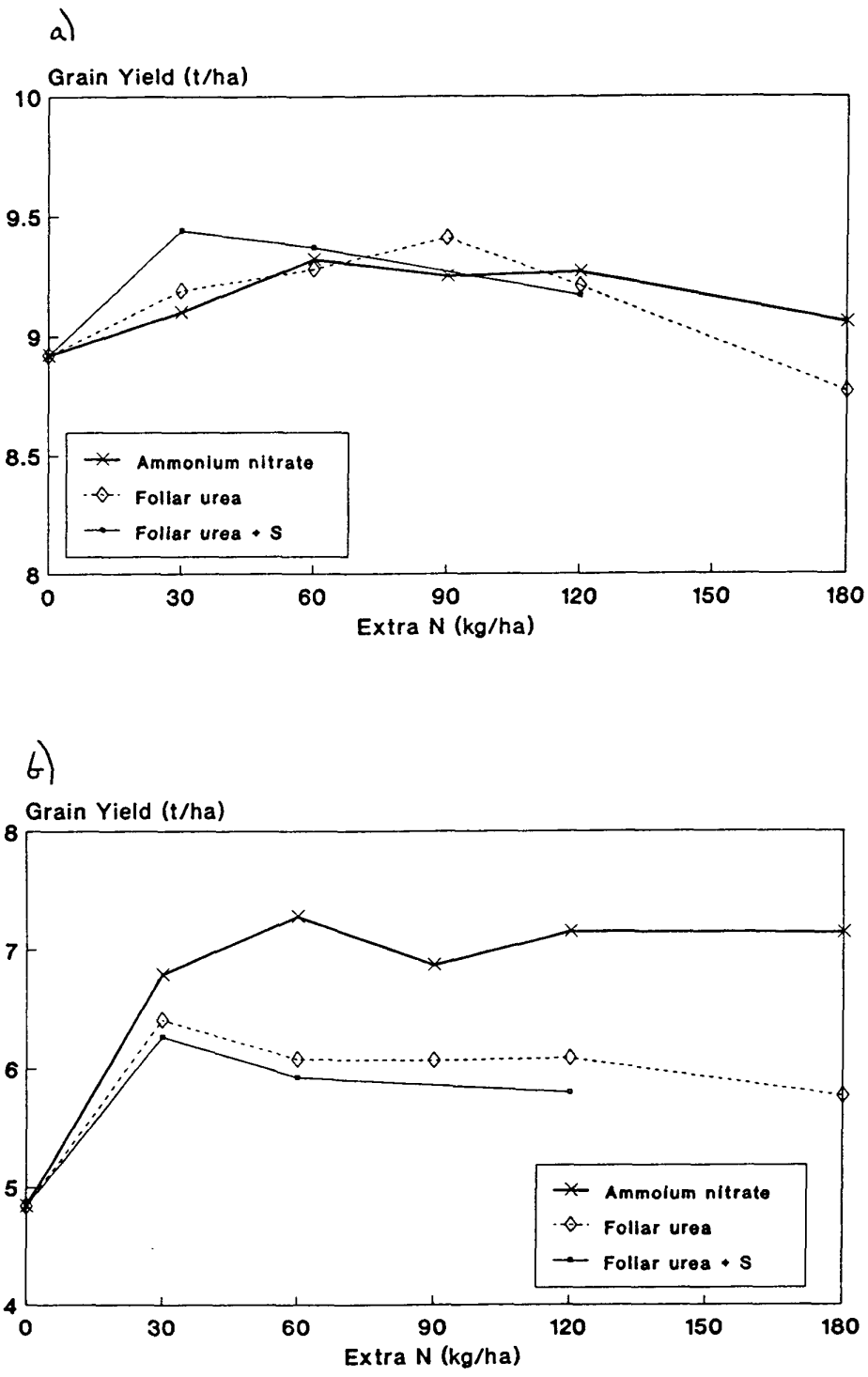


Figure 2. Examples of yield responses to extra N.
a) Equal response from both forms of N (High Mowthorpe 1990).
b) Higher yield from ammonium nitrate (Nocton 1988)

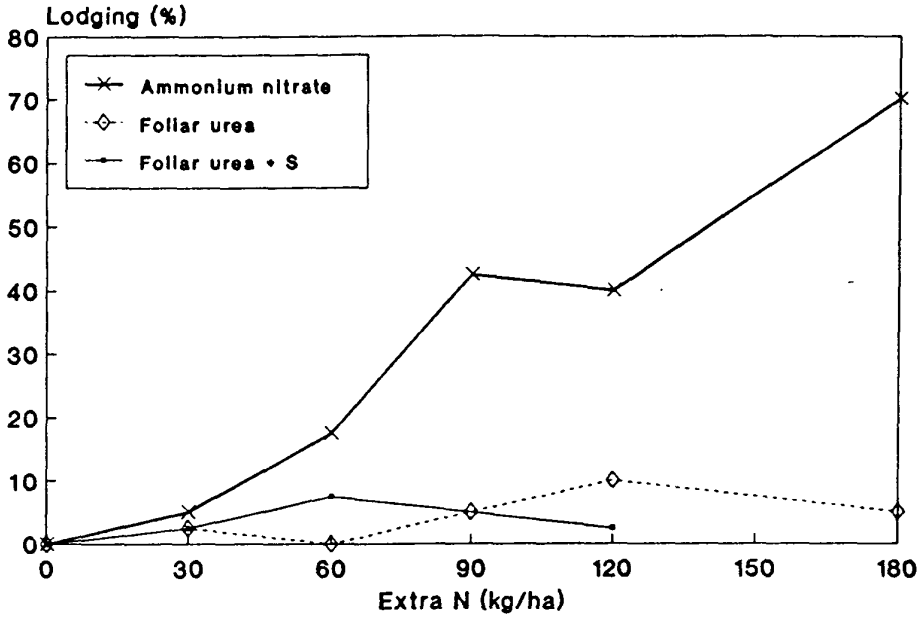


Figure 3. Effect of extra N on crop lodging (Bapton 1988).

5.1.2 Grain protein content (see Appendix 2 for site data).

At all 22 sites, use of extra N was a consistent and reliable means of increasing grain protein content. When meaned over all rates of application, there were significant differences ($p<0.05$) as follows:

a)	no effect from either form of N	0 sites
b)	equal increase from both forms of N	2 sites
c)	higher protein from ammonium nitrate	1 site
d)	higher protein from foliar urea	19 sites
e)	increase from increasing N rates	20 sites
f)	decrease from increasing N rates	0 sites
g)	increase from sulphur application	1 site
h)	decrease from sulphur application	3 sites

Large increases in protein were obtained at most sites up to the highest rate of extra N tested (180 kg/ha N), particularly when applied as foliar urea. The overall effect across all sites is shown in Figure 4. Although the rates of extra N tested were well above normal commercial

practice (c.40 kg/ha N), increases of over 3% grain protein were achieved at 10 sites and of over 4.5% protein at 1 site from use of the highest rate of extra N.

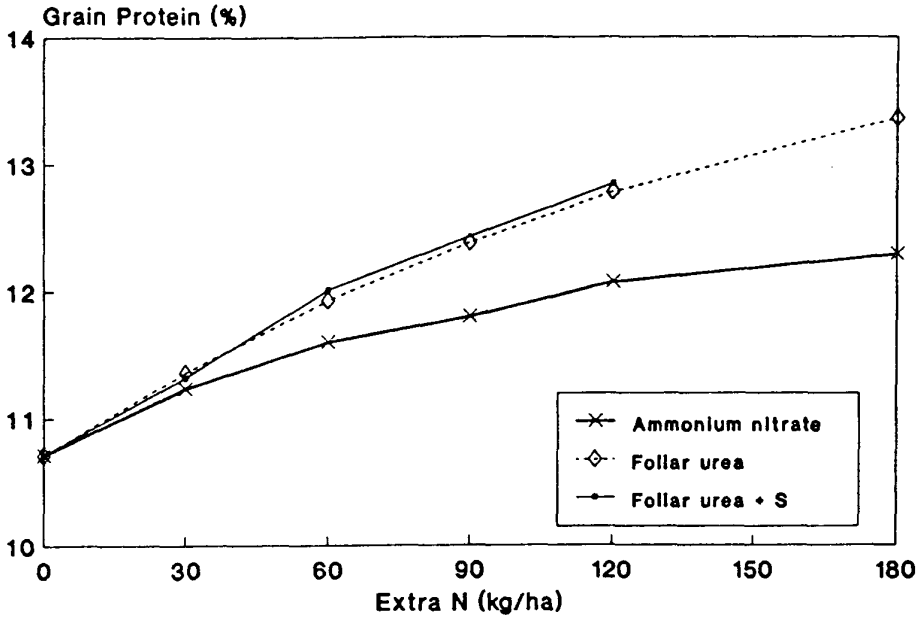


Figure 4. Mean effect of extra N on grain protein content (% at 86% DM) - mean of 22 sites

At nearly all sites (19 out of 22), extra N as foliar urea gave a higher grain protein content than as ammonium nitrate. For both forms of N, the rate of response of grain protein to extra N was approximately linear up to at least 60 kg/ha extra N but flattened off at higher rates. The rate of response was always higher from foliar urea than from ammonium nitrate.

The mean increase in grain protein from different levels of applied extra N are shown in Table 4; 30 kg/ha of extra N as ammonium nitrate increased grain protein by 0.51%, and by 0.66% when applied as foliar urea.

Table 4. Increase in grain protein content (% at 86% DM) from extra N applied as ammonium nitrate or foliar urea - mean of 22 sites, values from computer fitted curves.

Extra N (kg/ha)	Ammonium nitrate (GS32)	Foliar urea (GS75)	Foliar urea + S (GS75)
	(control protein content = 10.71%)		
30	0.51	0.66	0.61
60	0.88	1.21	1.30
90	1.14	1.68	-
120	1.33	2.07	2.14
180	1.59	2.65	-

However, for both forms of N the rate of response was influenced by the control protein content where no extra N was applied (Table 5). At sites where this was high, the rate of response of protein to extra N was low, and vice versa. This relationship is illustrated in Figures 5a, b and c for different levels of control grain protein content.

Across all sites, protein increases resulting from 30 kg/ha extra N as ammonium nitrate ranged from 0.17-0.60%, and for foliar urea from 0.40-0.71%, where the control grain protein content before application ranged from above 12.0% to below 10.5%. The response of protein to foliar urea was less sensitive to the control grain protein content than for ammonium nitrate.

Table 5. Increase in grain protein content (% at 86% DM) from 30 kg/ha extra N applied as ammonium nitrate or foliar urea according to the grain protein content in control treatments.

Control grain protein content	Ammonium nitrate (GS32)	Foliar urea (GS75)
Below 10.5% (10 sites)	0.60	0.71
10.5-12.0% (6 sites)	0.46	0.67
Over 12.0% (6 sites)	0.17	0.40

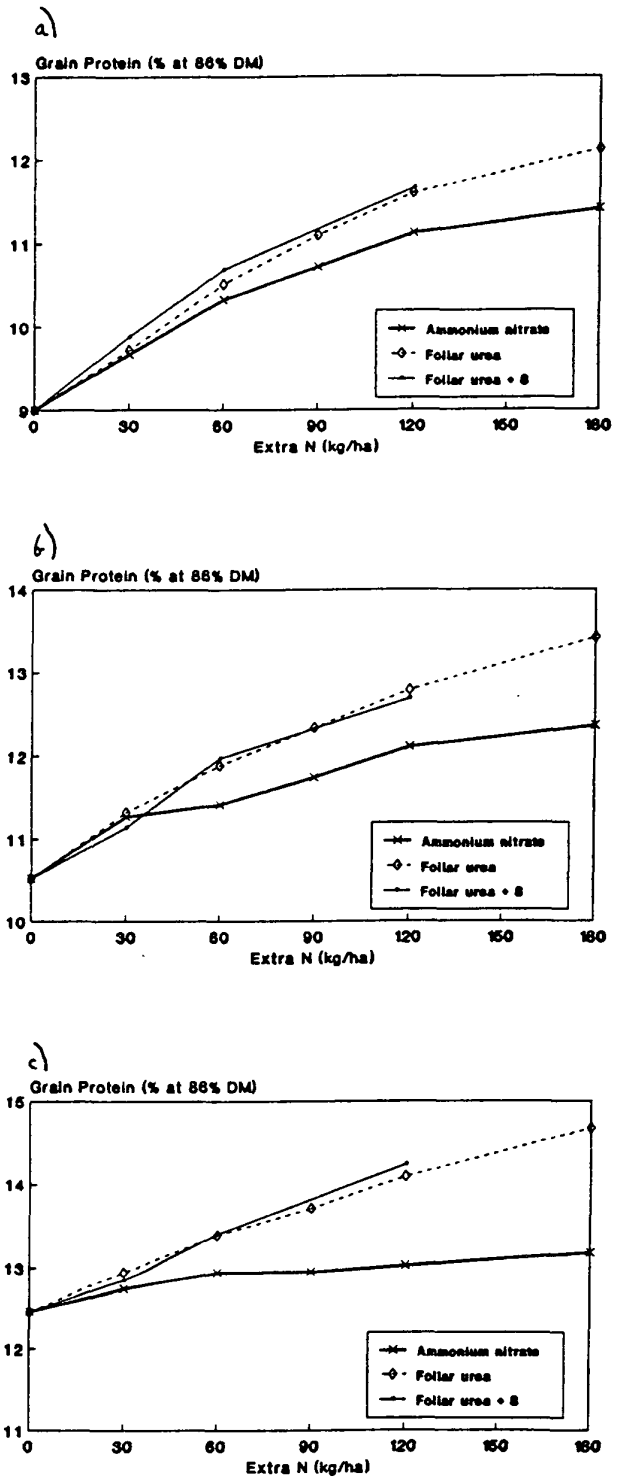


Figure 5. Effect of extra N on grain protein content at sites where:

(a) Control protein level below 10.5% (mean of 10 sites)
(b) Control protein level 10.5-12% (mean of 6 sites)
(c) Control protein level above 12.0% (mean of 6 sites)

Figure 4 clearly shows that there is a higher potential maximum grain protein content where foliar urea is used. At 12 sites (11 Mercia, 1 Avalon), there were significant interactions ($p<0.05$) between the rate and form of extra N used with foliar urea giving a larger protein response to extra N.

Computer fitting of the mean data shown in Figure 4 was carried out using a linear plus exponential function (Sylvester-Bradley et al, 1982) where x=extra N with constants $a>0$ and $b, c<0$ and $0<r<1$.

$$\text{Protein} = a + b*r^{**}x + c*x$$

Extrapolation from these fitted curves gives mean grain protein contents which might be regarded as potential maxima according to the form of extra N used. These values are shown below.

Extra N as ammonium nitrate	12.5%
Extra N as foliar urea	14.2%

The actual maximum values of grain protein content achieved were 14.1% (site 11, Bladon 1989, Mercia) and 15.3% protein (site 24, Milton 1991, Mercia) from extra N as ammonium nitrate and foliar urea respectively.

Sulphur application gave a significant increase in grain protein at 1 site but a significant decrease at 3 sites. At 3 of these sites, the reverse effect occurred for sulphur on yield. There were no differences in N recovery at these sites, which would indicate that sulphur was not influencing total N uptake but preferentially increasing yield rather than grain protein content.

5.1.3 Grain N offtake and the apparent % recovery of applied extra N in grain (see Appendix 3 for site data).

The offtake of N in grain depends on grain yield and grain protein content. Significant effects ($p<0.05$) on the apparent recovery of the applied extra N in grain were assessed at 22 sites as follows:

a)	no effect of form or rate of N	8 sites
b)	equal effect from both forms of N	7 sites
c)	higher recovery from ammonium nitrate	1 site
d)	higher recovery from foliar urea	6 sites
e)	increase from increasing N rates	0 sites
f)	decrease from increasing N rates	7 sites
g)	increase from sulphur application	1 site
h)	decrease from sulphur application	0 sites

Across all sites, there was a mean decrease in N recovery from increasing rates of extra N applied (Figure 6) - this effect was significant at 7 sites.

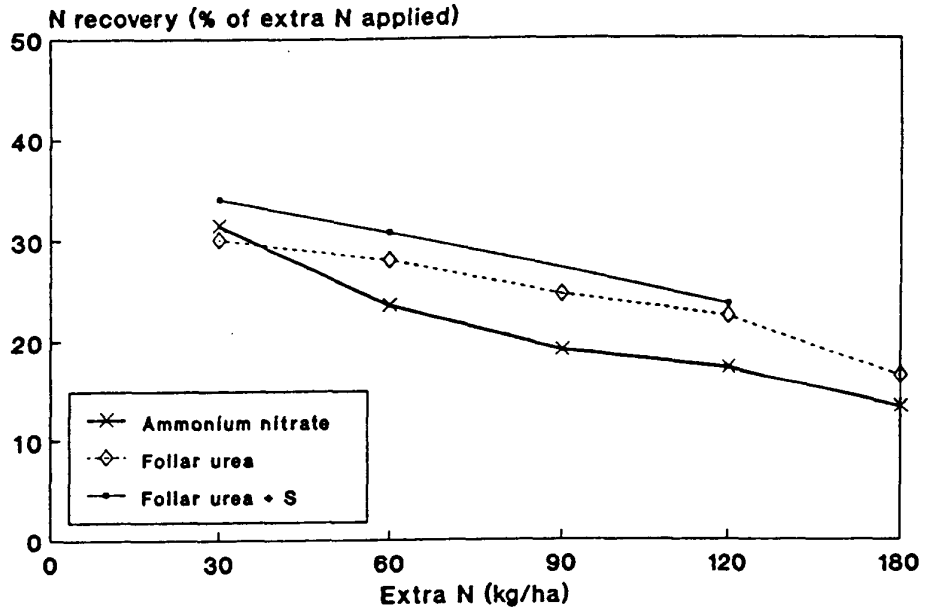


Figure 6. Apparent recovery of applied extra N in grain - mean of 22 sites (Trial Series A).

Overall, N recovery was slightly higher where extra N was applied as foliar urea. Although the differences were small, it does indicate that application of extra N as foliar urea at GS75 is usually no less efficient than earlier applications as ammonium nitrate, and is commonly more efficient.

At 7 sites, there was a mean difference between the 2 forms of N. At 6 sites, extra N applied as foliar urea gave a significantly higher N recovery ($p<0.05$). At 1 site, N recovery was higher from ammonium nitrate.

The actual recovery of applied extra N varied considerably between sites, but was low even at the low rates of N application. When meaned over all sites, about 30% of the N applied at the 30 kg/ha N rate was recovered in the grain. This means that 70% of the application (21 kg/ha N) remained as a residue in the soil/crop debris following harvest, thus being susceptible to loss from the soil/plant system by leaching of nitrates or other mechanisms. At higher rates of extra N, this residue level was much higher reaching 87%, or 156 kg/ha from an application of 180 kg/ha extra N as ammonium nitrate (Table 6).

These recovery values can be compared to the typical recovery of 50% of a normal spring rate of nitrogen applied for yield.

Table 6. Quantity of applied N recovered in grain and left as soil/crop residue following application of extra N applied as ammonium nitrate or foliar urea - mean of 22 sites.

Extra N (kg/ha)	Ammonium nitrate (GS32)		Foliar urea GS75)		Foliar urea + S (GS75)	
	Recovered	Residue	Recovered	Residue	Recovered	Residue
30	9.5	20.5	9.0	21.0	10.2	19.8
60	14.1	45.9	16.8	43.2	18.5	41.5
90	17.1	72.9	22.1	67.9		
120	20.7	99.3	26.8	93.2	28.4	91.6
180	24.0	156.0	29.6	150.4		

Figure 7 illustrates data from both Trial Series and shows that the extremely wide range of nitrogen recoveries between sites was strongly influenced by the level of grain protein in the control plots (r^2=49.4%). Nitrogen recovery ranged from nil up to 75% of N applied as foliar urea, and was higher where the control level of grain protein content was low, reflecting the larger capacity of the grain to increase protein. The relationship was similar for extra N applied as ammonium nitrate.

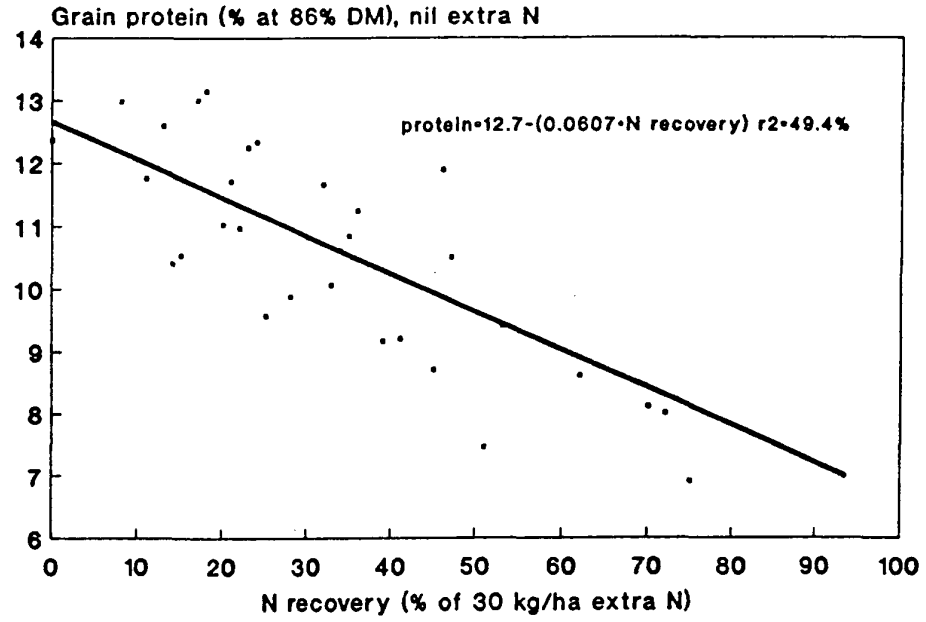


Figure 7. Apparent recovery in grain of 30 kg/ha extra N applied as foliar urea according to the grain protein content of control treatments (Trial Series A and B, 44 sites).

In the critical range of 10.5 to 11.0% grain protein, the recovery of 30 kg/ha extra N was between 30-35% - ie. a residue of 20 kg/ha N was left in the soil and crop debris.

5.1.4 Grain SDS sedimentation volume (see Appendix 4 for site data).

The effects of extra N on grain SDS were much less than the effects on grain protein content. Significant effects at 21 sites ($p<0.05$) were as follows:

a)	no effect from either form of N	10 sites
b)	equal increase from both forms of N	4 sites
c)	higher SDS from foliar urea	6 sites
d)	higher SDS from ammonium nitrate	1 site
e)	increase from sulphur application	3 sites
f)	decrease from sulphur application	0 sites

SDS values varied widely between sites partly influenced by the protein content at individual sites. However, the large responses of grain protein to the extra N treatments were generally not reflected in changes in grain SDS.

The overall effect of extra N on SDS values is shown in Figure 8. Extra N as foliar urea had a larger effect than as ammonium nitrate, reflecting the effect of this treatment on grain protein content. However, the size of the effect on SDS was small and probably within the variability associated with the method of analysis - 60 kg/ha extra N as foliar urea gave a mean increase of only 2.5 mls.

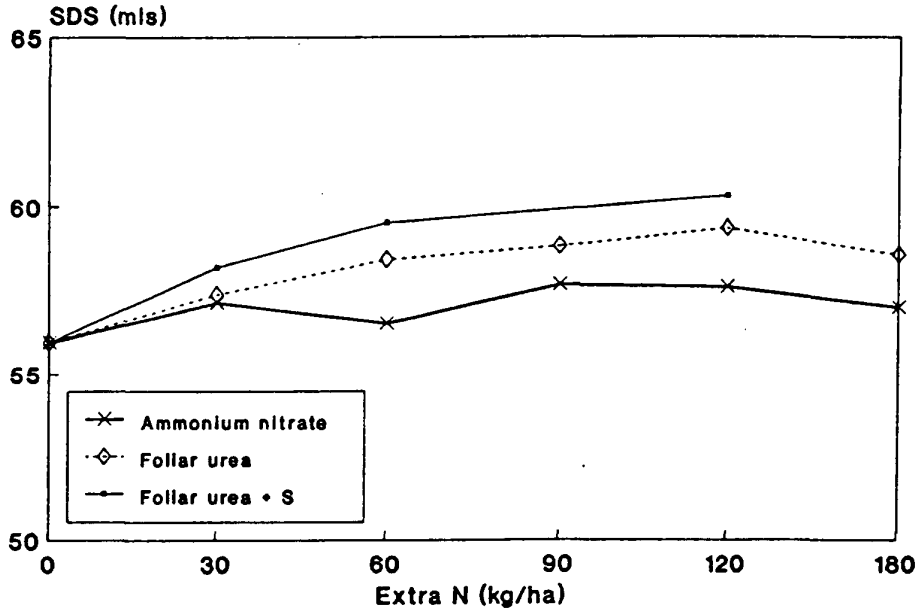


Figure 8. Mean effect of extra N on grain SDS (mls) - mean of 21 sites.

Use of sulphur had a small overall additional benefit, and gave a significant increase in SDS at 4 sites (range 1.0-6.7 mls, mean 2.9 mls). At one of these sites (site 8, Hargrave 1989), there were significant increases in both grain protein content and SDS.

5.1.5 Hagberg Falling Number (HFN, see Appendix 5 for site data).

HFN data were available from 21 sites. There were significant effects ($p<0.05$) of nitrogen treatment as follows:

a)	no effect from either form of N	10 sites
b)	equal increase from both forms of N	0 sites
c)	equal decrease from both forms of N	1 site
d)	higher HFN from foliar urea	10 sites
e)	higher HFN from ammonium nitrate	0 sites
f)	increase from sulphur application	0 sites
g)	decrease from sulphur application	1 site

At 10 sites, extra N had no effect on HFN. However, at another 10 sites foliar urea treatment gave an apparent increase, though the magnitude of effects were small - the average increase from foliar urea was 17 seconds (range 9-27 seconds) when meaned over all rates of N.

Although of interest, these small apparent effects of extra N on HFN are unlikely to influence decisions on the use of extra N, particularly since it is not possible to establish if the effects found in these trials are due to real differences or differences in crop maturity at harvest due to the different nitrogen treatments that were applied. Since all trial plots at a given site were harvested on the same day, it is possible that the apparent small benefit of foliar urea may have been due to treatment-induced differences in crop maturity rather than any real effect.

There was no benefit from sulphur application at any site.

5.1.6 Specific weight (see Appendix 6 for site data).

Specific weight data were available from 18 sites. Significant treatment effects ($p<0.05$) were infrequent and small - the size of these effects was usually less than 1.5 kg/hectolitre.

a)	no effect from either form of N	7 sites
b)	equal increase from both forms of N	1 sites
c)	equal decrease from both forms of N	2 sites
d)	higher sp. wt from foliar urea	5 sites
e)	higher sp. wt. from ammonium nitrate	3 sites
f)	increase from sulphur application	0 sites
g)	decrease from sulphur application	3 sites

Although there were some significant treatment effects on specific weight, the size of the effects was always small (less than 1.5 kg/hl) and inconsistent. At 5 sites specific weight was higher where extra N was applied as foliar urea, and at 2 sites higher where ammonium nitrate was used.

Sulphur application gave a significant reduction in specific weight at 3 sites, but by less than 1.0 kg/hl.

5.2 Trial Series B. Effect of timing of foliar urea application.

Because the timing treatments were applied on a chronological basis (every 10 days from GS 39), it is not possible to analyse the data across all sites to produce a **balanced** mean according to Growth Stages. However, an indication of the overall effect of timing according to Growth Stages can be obtained by meaning responses according to the time of application within Growth Stage bands to produce an **unbalanced** mean. Data are presented in this way for each parameter.

5.2.1 Grain yield and lodging (see Appendix 8 for site data).

Significant responses ($p<0.05$) for the effect of foliar urea timings on grain yield can be assessed at 23 sites as follows:

a)	no effect from any timing	13 sites
b)	increase from one or more timings	8 sites
c)	decrease from one or more timings	2 sites
d)	increase from sulphur application	0 sites
e)	decrease from sulphur application	0 sites

At most sites there was no significant effect of foliar urea on grain yield.

At 8 sites, foliar urea increased yield by a mean of 0.39 t/ha (range 0.15-1.08 t/ha). However, apart from site 26 (Brampton 1988) all yield increases were less than 0.40 t/ha. At 4 of these sites, the base level of N was between 40-100 kg/ha lower than normal recommendations (due to the design of the trial - see section 3), and it is assumed that this is the main reason for these responses.

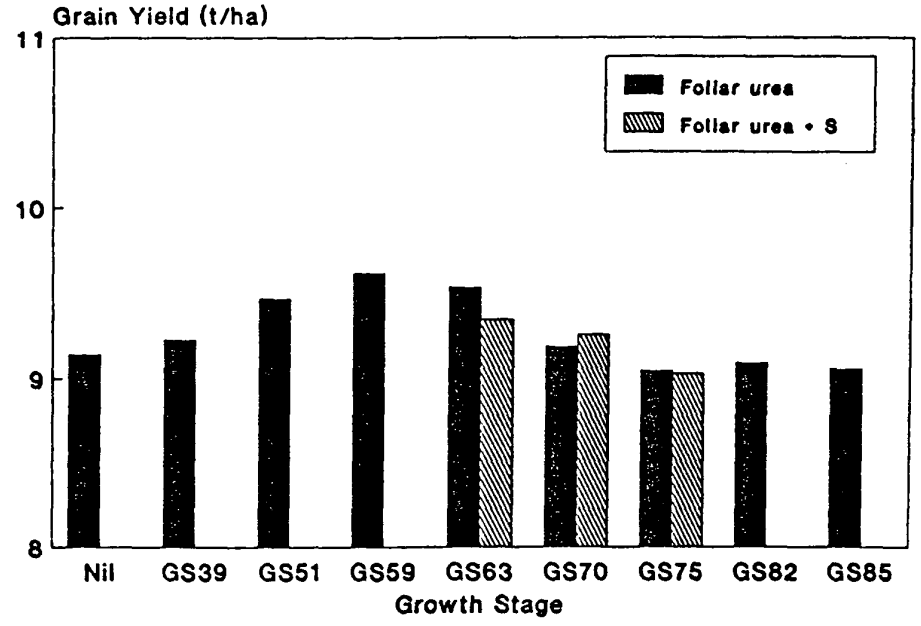


Figure 9. Effect of timing of application of foliar urea on grain yield (Rosemaund 1991)

At 6 of these 8 sites, there were significant differences between urea timings. The optimum timings for grain yield were mostly in the range GS 43 (early booting) to GS 65 (mid anthesis) - see Appendix 8. Later applications did not usually increase yield. Figure 9 illustrates the effect of different foliar urea timings at a responsive site.

At 2 sites, a yield decrease due to foliar urea was apparent. At site 35 (ADAS Boxworth, 1989), there was a mean yield reduction of 0.53 t/ha across all timings. At site 47 (Milton, 1991), there was a yield reduction from foliar urea applied at GS 39 and 73 which was associated with a higher level of flag leaf scorch from these treatments.

The overall effect of urea timings based on **unbalanced** means of all site/treatment data is shown in Table 7.

Table 7. Mean response of grain yield according to time of application of foliar urea (40 kg/ha N or 30 kg/ha N at sites 27-30) - unbalanced mean of all site/treatment responses.

Time of application (GS)		Number of datapoints	Mean yield response (t/ha)
GS 39	Flag leaf emerging	20	+0.03
GS 40-49	Booting	16	-0.03
GS 50-59	Ear emergence	23	+0.14
GS 60-69	Anthesis	30	+0.08
GS 70-79	Milk development	41	+0.09
GS 80-89	Dough development	39	+0.02
GS 90-95	Ripening	9	-0.04
Mean of all timings		178	+0.03

There were 2 sites which gave responses to sulphur application that were very close to statistical significance. At site 39 (ADAS Boxworth, 1990), sulphur reduced yield by 0.11 t/ha ($p=0.055$), whilst at site 47 (Milton, 1991), sulphur increased yield by 0.07 t/ha ($p=0.054$).

5.2.2 Grain protein content (see Appendix 9 for site data).

Significant responses ($p<0.05$) for the effect of foliar urea treatment on grain protein content were assessed at 23 sites as follows:

a)	no effect from any timings	4 sites
b)	increase from one or more timings	19 sites
c)	decrease from one or more timings	0 sites
d)	increase from sulphur application	0 sites
e)	decrease from sulphur application	0 sites

Not unexpectedly, foliar urea increased grain protein at most sites. The mean increase in protein across all timings was 0.34% from 40 kg/ha urea-N. When applied between GS70-79, however, the mean increase was 0.71% protein. As for Trial series A, there was a range in responsiveness that was largely due to variation in the control protein level of each site, with larger responses occurring at lower levels of protein. However, other unspecified factors influence responsiveness, since the most responsive site had a control protein level of 12.23% (site 42, ADAS Rosemaund 1991).

Estimates of the optimum growth stage for foliar urea application for grain protein varied considerably between sites (see Appendix 9). The unbalanced mean responses from foliar urea applied within Growth Stage bands is shown in Table 8.

Table 8. Mean response of grain protein content according to time of application of foliar urea (40 kg/ha N or 30 kg/ha N at sites 27-30) - unbalanced mean of all site/treatment responses.

Time of application (GS)		Number of datapoints	Protein response (%)
GS 39	Flag leaf emerging	20	+0.34
GS 40-49	Booting	16	+0.41
GS 50-59	Ear emergence	23	+0.59
GS 60-69	Anthesis	30	+0.42
GS 70-79	Milk development	41	+0.71
GS 80-89	Dough development	39	+0.32
GS 90-95	Ripening	9	+0.10
Mean of all timings		178	+0.34

From this analysis, and inspection of individual site data, the optimum timing of foliar urea application was during milk development (GS 70-79), giving a mean increase of 0.7% protein from an application of 40 kg/ha urea-N. This is comparable to the mean protein increase found in Trial Series A. Figure 10 shows a typical response of grain protein to different timings of foliar urea application. At some sites, some earlier timings were equally effective, but application during milk development (GS 70-79) nearly always gave close to the maximum response. Foliar urea applied after milk development was generally much less effective.

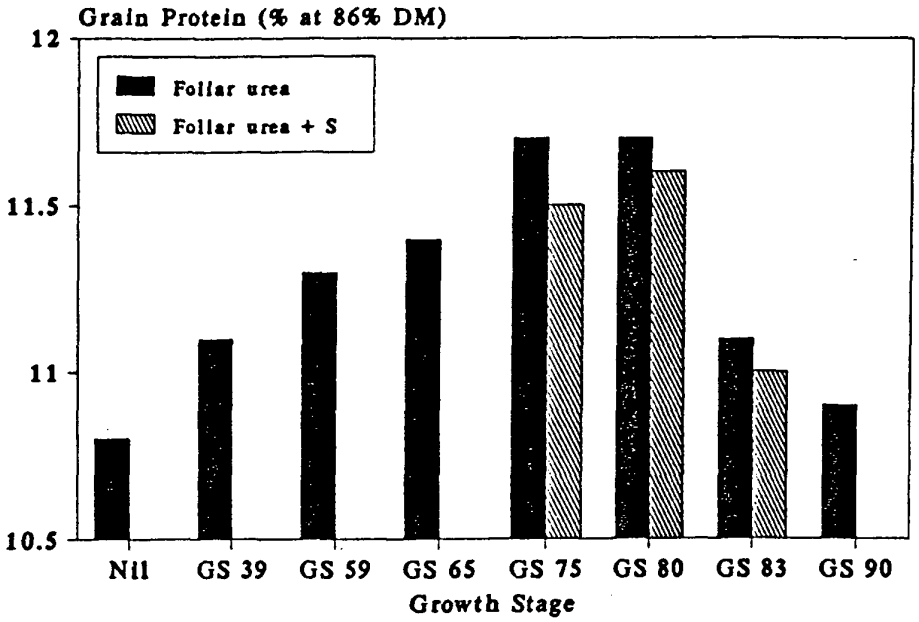


Figure 10. Effect of timing of application of foiliar urea on grain protein content (Rosemaund 1988)

5.2.3 Grain N offtake and the apparent % recovery of applied extra N in grain (see Appendix 10 for site data).

Significant responses ($p<0.05$) for the apparent recovery of the extra N applied as foliar urea was assessed at 23 sites as follows:

a)	no difference between any timings	6 sites
b)	difference between one or more timings	17 sites
c)	increase from sulphur application	1 site
d)	decrease from sulphur application	1 site

Across all sites and timings, the mean recovery of extra N was only 11%. However, largely due to differences in the level of control grain protein, individual site data given in Appendix 10 show that there was a very wide variation in the mean recovery across all foliar urea timings ranging from 0 up to 69% recovery.

At most sites, timing of urea application had a significant effect on the apparent recovery of the applied extra N, but there was considerable variation in the optimum timing between sites. The overall effect of different timings is shown by the unbalanced means in Table 9.

Table 9. Mean apparent recovery of applied extra N according to time of application of foliar urea (40 kg/ha N or 30 kg/ha N at sites 27-30) - unbalanced mean of all site/treatment responses.

Time of application (GS)		Number of datapoints	Recovery of applied N (%)
GS 39	Flag leaf emerging	20	+11
GS 40-49	Booting	16	+21
GS 50-59	Ear emergence	23	+26
GS 60-69	Anthesis	30	+20
GS 70-79	Milk development	41	+28
GS 80-89	Dough development	39	+10
GS 90-95	Ripening	9	+ 8
Mean of all timings		178	+11

Urea timing between GS 70-79 gave the highest overall N recovery, reflecting the optimum effect of this timing on grain protein. Recovery of extra N was notably poorer from either very early (GS 39) or very late applications (after GS 80) reflecting the poor utilisation of these applications for either grain yield or protein.

5.2.4 Grain SDS sedimentation volume (see Appendix 11 for site data)

Significant responses ($p<0.05$) of grain SDS volume to foliar urea treatment were assessed at 21 sites as follows:

a)	no effect from any timing	11 sites
b)	increase from one or more timings	9 sites
c)	decrease from one or more timings	3 sites
d)	increase from sulphur application	0 sites
e)	decrease from sulphur application	0 sites

Overall, foliar urea had little effect on grain SDS - where responses occurred, they were very small and well below 2 mls. The large effects of foliar urea application on grain protein contents were not generally mirrored by any significant change in SDS levels. Of the 19 sites where foliar urea gave a significant increase in grain protein, only 7 sites gave a corresponding increase in grain SDS.

At 2 sites there was a decrease in SDS - at site 45 (Bethesden, 1991) the decrease in SDS was associated with no effect on grain protein; at site 31 (Rosemaund, 1989), foliar urea increased grain protein but decreased grain SDS.

Sulphur application had no effect on grain SDS at most sites - at site 44 (Boxworth, 1991) sulphur gave an indication of a decrease in SDS but this effect was not quite statistically significant (p=0.051).

Based on unbalanced means, the overall effect of different timings of foliar urea is shown in Table 10. Although firm conclusions cannot be drawn because of the small size of the apparent responses, urea application between GS 60-79 appeared to have the maximum effect on SDS.

Table 10. Mean response of grain SDS according to time of application of foliar urea (40 kg/ha N or 30 kg/ha N at sites 27-30) - unbalanced mean of all site/treatment responses.

Time of application (GS)		Number of datapoints	Grain SDS (mls)
GS 39	Flag leaf emerging	19	+1.3
GS 40-49	Booting	15	+0.3
GS 50-59	Ear emergence	22	+0.9
GS 60-69	Anthesis	29	+1.5
GS 70-79	Milk development	38	+1.8
GS 80-89	Dough development	38	-0.5
GS 90-95	Ripening	9	+0.4
Mean of all timings		170	+1.3

5.2.5 Hagberg Falling Number (HFN, see Appendix 12 for site data).

Significant responses ($p<0.05$) of grain HFN to foliar urea treatment were assessed at 22 sites as follows:

a)	no effect from any timing	16 sites
b)	increase from one or more timings	6 sites
c)	decrease from one or more timings	0 sites
d)	increase from sulphur application	2 sites
e)	decrease from sulphur application	1 site

Although Hagbergs were apparently increased by foliar urea at 6 sites, the increases were generally small - mean +23, maximum +42. Although there were significant differences due to urea timing at 3 of these 6 sites it was not possible to identify an overall optimum range for urea timing.

Sulphur application increased Hagbergs at 2 sites, but reduced Hagbergs at a third. Effects due to sulphur were all less than +/- 20.

It is not possible to say if these responses were due to differences in crop maturity at harvest due to treatment application or due to some other physiological effect.

5.2.6 Specific weight (see Appendix 13 for site data).

Significant responses ($p<0.05$) of grain specific weight to foliar urea treatments were assessed at 19 sites as follows:

a)	no effect from any timing	14 sites
b)	increase from one or more timings	5 sites
c)	decrease from one or more timings	0 sites
d)	increase from sulphur application	0 sites
e)	decrease from sulphur application	3 sites

All specific weight responses were very small and less than 1.1 kg/hl. At 4 sites, foliar urea increased specific weight and at 3 of these there was a significant difference between urea timings. In general at these sites, late timings had little effect on specific weight.

6. RESULTS - MILLING AND BAKING QUALITY

A total of 8 sites from trial Series A, and 9 sites from Trial Series B were analysed for milling and baking quality (see Table 3 for details). A full set of data and statistics is given in Appendices 16 and 17.

6.1 Trial Series A. Effect of rate of extra N as ammonium nitrate (GS 32) or foliar urea (GS 75).

6.1.1 Milling quality

Milling quality was assessed in terms of the extraction of white flour obtainable under a standard milling regime, and the colour (ie. level of bran contamination) measured in the resultant flour. The most desirable combination is a high flour extraction value and a low flour colour value.

Milling quality was generally considered to be good over all sites and seasons. The flour extraction rate and flour colour values for control plots, averaged over each of the four years of experimentation, are as follows:

Table 11. Average flour extraction rate and colour of Buhler-milled white flour produced (Trial Series A).

Year	Extraction rate (%)	Flour colour grade (GCF units)
1988	75.0	1.62
1989	77.4	-1.00
1990	75.4	-1.43
1991	79.8	2.62

Flour yields, obtained from the Buhler experimental mill, were somewhat lower than the commercial practice of the time and flour colour grades were generally exceptionally good. Normally a miller will produce white flour to a flour colour specification of around 2.5 colour grade units and this was easily achieved at most sites. An exception occurred at Bishops Canning

1991, where high levels of extra N resulted in some exceptionally high flour colour grades. Substantial differences occurred in both flour yield and flour colour grade between seasons, probably due to differences in grain plumpness and cleanliness resulting from the growing and harvesting conditions of each season.

For the 8 sites examined, milling performance was significantly affected ($p < 0.05$) by one or more of the treatments in the following manner:

A. Flour extraction rate

a)	No effect due to either form of extra N.	4 sites
b)	Increase in extraction rate from ammonium nitrate.	1 site
c)	Decrease in extraction rate from foliar urea.	1 site
d)	No effect of sulphur	6 sites

B. Flour colour grade

a)	No effect due to either form of extra N.	0 sites
b)	Increase in flour colour from ammonium nitrate	8 sites
c)	Increase in flour colour from foliar urea.	8 sites
d)	Increase from increasing N rates.	8 sites
e)	Higher flour colour from foliar urea.	3 sites
f)	No effect of sulphur	8 sites

Flour extraction rate was relatively stable within this trial series and, as indicated above, there was no overall significant effect of either fertiliser treatment. Figure 11 for Hargrave 1989 shows a general increase in flour extraction rate with increasing rates of ammonium nitrate-N. This relationship is not typical as in all other cases treatment had no effect on this parameter. However, it is obvious that even in this case changes in flour extraction were inconsequential when viewed against the background of the between-harvest differences shown in Table 11.

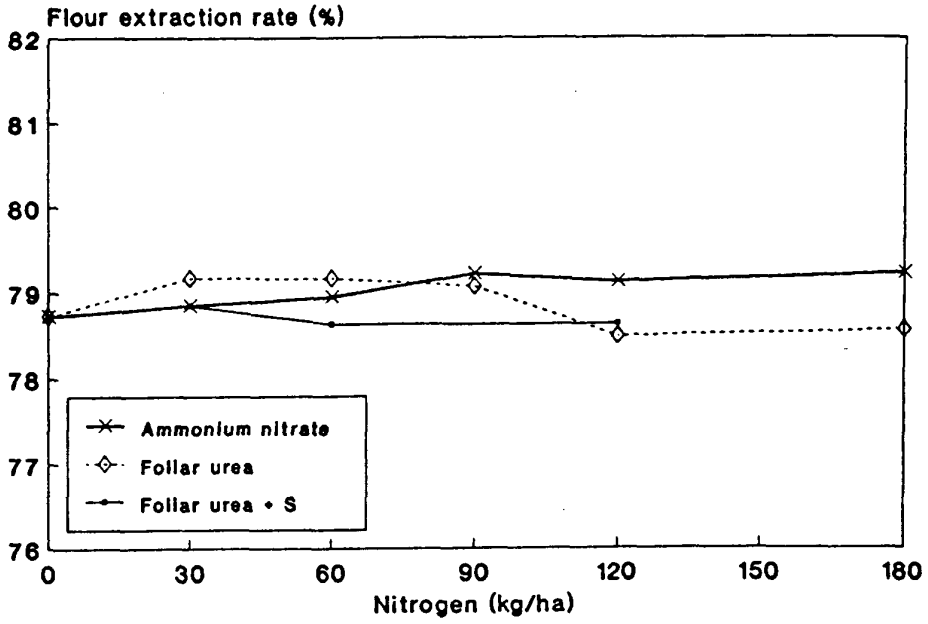


Figure 11. Effect of extra N on flour extraction rate (Hargrave 1989)

Coefficients of variation were generally high for flour colour measurements, but despite this, significant upward trends were observed with increasing rates of both ammonium nitrate, and more particularly, foliar urea. Figure 12 shows a typical trend in flour colour with increasing rates of extra N for the Roundway site in 1990. To put such an effect into context, the majority of trials examined at FMBRA had negative mean colour grade values. Therefore observed increases in flour colour would not generally result in the miller reducing flour extraction in order to maintain colour values within a breadmaking colour specification of around 2.5 grade colour units.

6.1.2 Flour protein content

The effect of N treatments on grain protein content is discussed in Section 5 of this report. However, since flour protein content has a direct effect on the final breadmaking performance of a particular variety and, furthermore, since flour protein content can be influenced by the losses which occur on flour milling this parameter was further investigated with regard to its effect on breadmaking quality.

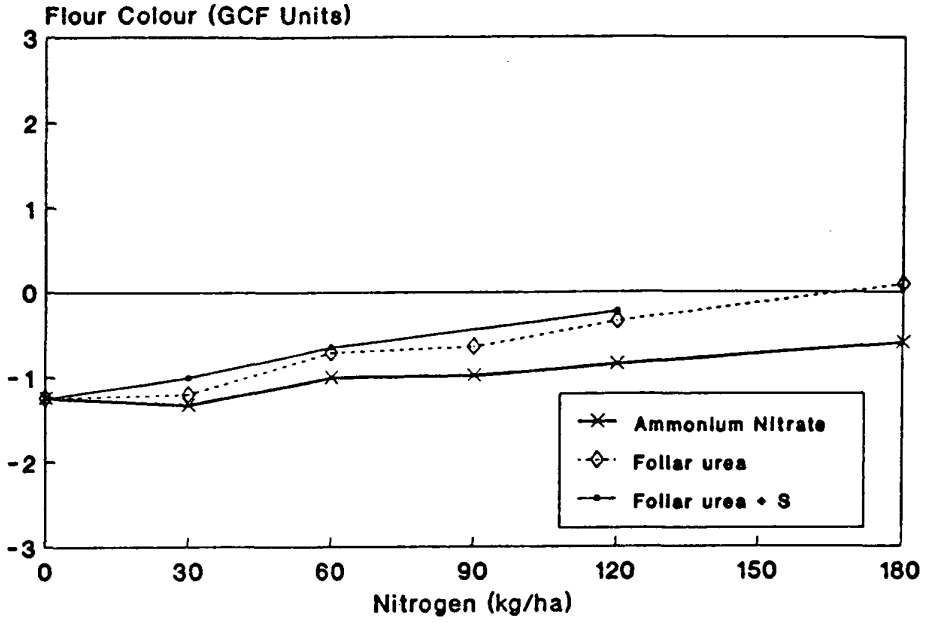


Figure 12. Effect of extra N on flour colour (Roundway 1990)

In addition, grain protein content was one of the major determinants used in the selection of samples for quality assessment. Therefore, there is a slight bias to the results presented under this heading. Results presented in Section 5.1.2 show overall effects of treatments on protein content.

Average protein losses upon milling are shown in Table 12 for trial sites examined at FMBRA - values are calculated by subtracting flour protein values (analysed by FMBRA) from grain protein values (analysed by ADAS).

Table 12. Mean flour protein content and protein loss upon milling (% at 86% DM) for control treatment, 180 kg/ha N as ammonium nitrate and 180 kg/ha N as foliar urea (Trial Series A).

Site	Control		Ammonium nitrate		Foliar urea	
	Mean flour prot. %	Mean prot. loss %	Mean flour prot. %	Mean prot. loss %	Mean flour prot. %	Mean prot loss %
1988						
Kneesall	7.80	1.4	10.20	1.4	11.30	1.7
1989						
Boyt	9.70	0.83	10.62	1.36	11.05	1.44
Folk/ham	9.25	0.62	10.90	0.60	11.62	0.80
Hargrave	8.50	0.66	10.80	0.59	11.55	0.57
1990						
Frampton	8.28	0.61	10.30	0.78	10.13	1.27
Roundway	9.20	0.36	10.83	1.12	11.83	1.17
1991						
Bishops Canning	10.30	1.58	11.60	1.63	14.10	1.58
Milton Earnest	10.70	1.62	12.00	1.07	13.80	1.52

Flour protein content was related to wheat protein content and changes in flour protein content therefore tended to mirror those observed for grain protein content. However, since protein content is higher in the outer layers of the grain, a loss of protein content on milling is to be expected. The extent of this loss appeared to depend on the year and trial site. The average protein loss on milling in this work was 1.1%, with some evidence of slightly higher losses when high levels of extra N were applied as ammonium nitrate or more particularly foliar urea.

The average flour protein content for the control treatment was unacceptable for breadmaking purposes at 7 of the trial sites selected (ie. below 10.5%, the commercial specification for breadmaking flour).

For all 8 sites examined, flour protein content was increased when extra N was applied. Significant responses ($p<0.05$) were assessed as follows:

a)	Increase in flour protein content	8 sites
b)	Foliar urea more effective in increasing flour protein content (ie comparing protein meaned over all rates)	4 sites
c)	Ammonium nitrate more effective in increasing flour protein content (comparison as in b)	0 sites
d)	No difference in the performance of N fertiliser treatments with regard to flour protein content (as b)	4 sites
e)	Increase due to increasing N rates.	8 sites
f)	High levels of foliar urea more effective than ammonium nitrate (comparing 180kgN/ha)	8 sites
g)	No effect of sulphur.	8 sites

As for grain protein content, substantial increases in flour protein content were observed at most sites up to the highest level of applied nitrogen fertiliser with foliar urea being more effective in this respect than ammonium nitrate.

Extra N successfully produced flour with a protein content in excess of 10.5% at all 8 sites. The rate of extra N required to achieve this target flour protein content at each site is given in Table 13.

Table 13. Amount of N fertiliser required at each site to produce flour of at least 10.5% protein content (at 86% DM).

Site	Ammonium nitrate (kg/ha N)	Urea (kg/ha N)
1988		
Kneesall	>180	120
1989		
Boyt	180	120
Folkingham	90	90
Hargrave	120	120
1990		
Frampton	>180	180
Roundway	120	90
1991		
Bishops Canning	0	0
Milton Ernest	0	0

Thus, in 3 of the 8 trials examined it was possible to obtain the required flour protein content using less foliar N than ammonium nitrate, and in both trials examined in 1991, flour protein levels above 10.5% could be achieved without additional N input.

Below 90 kg/ha extra N, the flour protein response curves were generally parallel with signs of divergence between the two forms of nitrogen at higher rates of N. In particular, the ammonium nitrate curve tended to flatten off at high N rates (90 kg/ha N and above) whereas the foliar urea curve continued to rise and generally started to show the first signs of diminishing protein returns at N rates above 120 kg/ha N. A typical response curve is shown in Figure 13.

Extra N, as foliar urea, appeared capable of producing continued increases in flour protein content up to values of over 14.0% at some sites. In addition, foliar urea consistently produced significantly higher flour protein content at the 180 kg/ha N rate of application than ammonium nitrate.

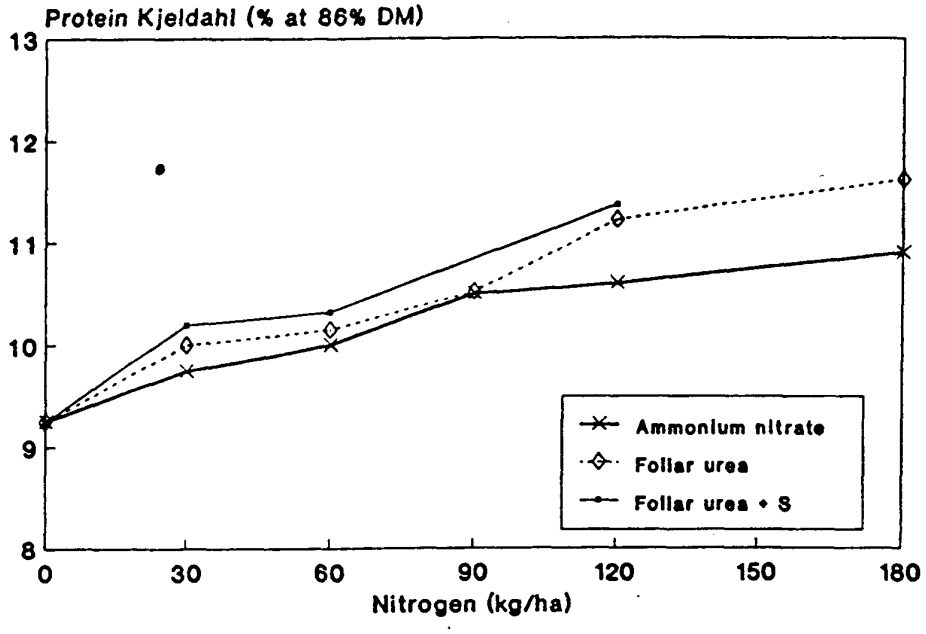


Figure 13. Effect of extra N on Kjeldahl protein (Folkingham 1989)

The response of flour protein to extra N was greater at low levels of control flour protein. This effect was consistent for both forms of applied nitrogen. Where the base nitrogen treatment produced a high flour protein content, the response to extra N tended to be reduced. A comparison of the different response curves for the Hargrave and Boyt (low and high protein sites in 1989) is shown in Figures 14a and b.

Sulphur treatments had no significant effect on flour protein content. Although sulphur treatment consistently gave higher flour protein contents than the equivalent urea only treated plots, the effect was never large enough to reach statistical significance. Mean differences ranged from 0.07 to 0.30% flour protein.

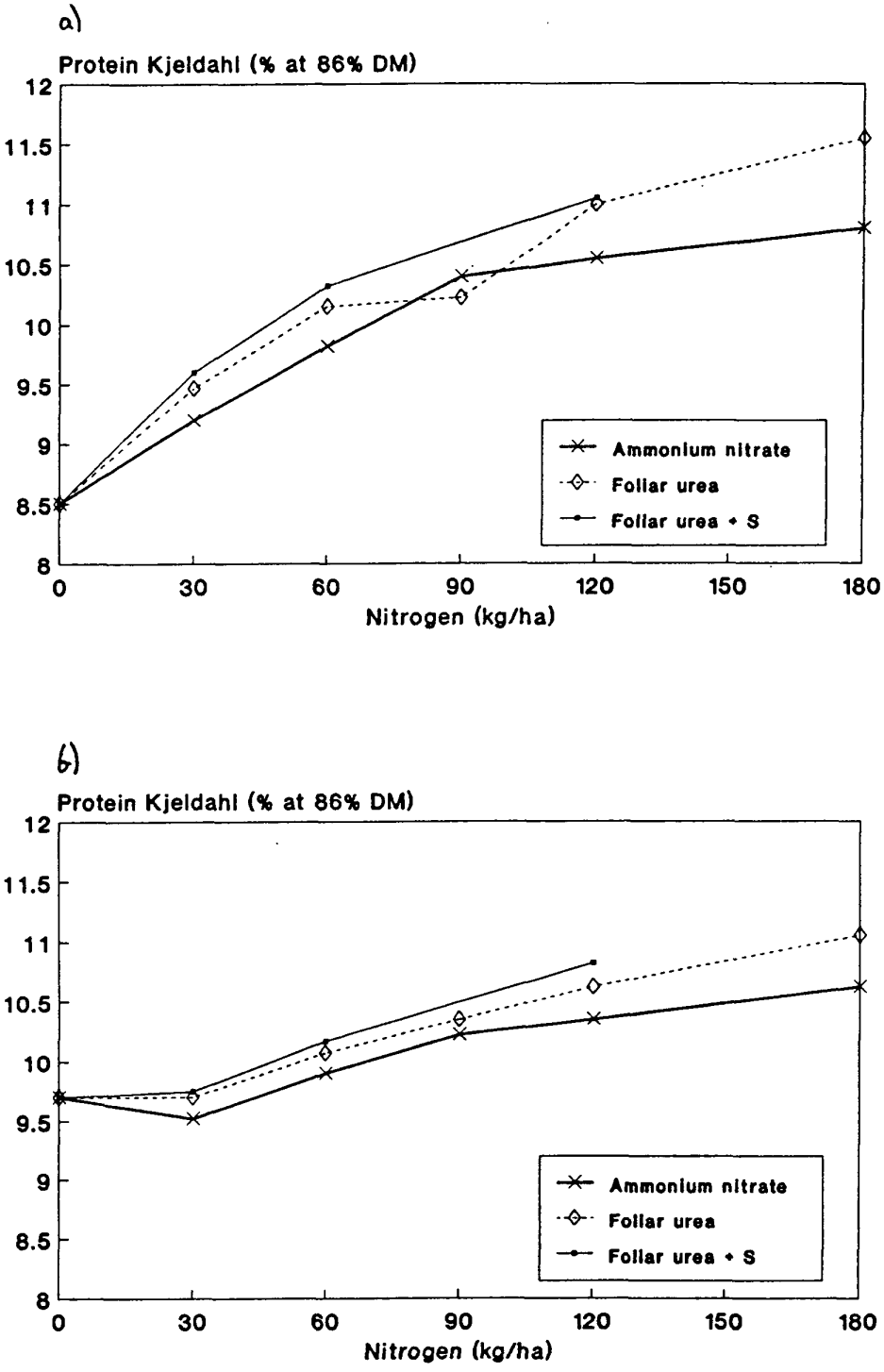


Figure 14. Comparison of flour protein response to extra N according to base protein level:

a) Low base level of protein (Hargrave 1989)
b) High base level of protein (Boyt 1989)

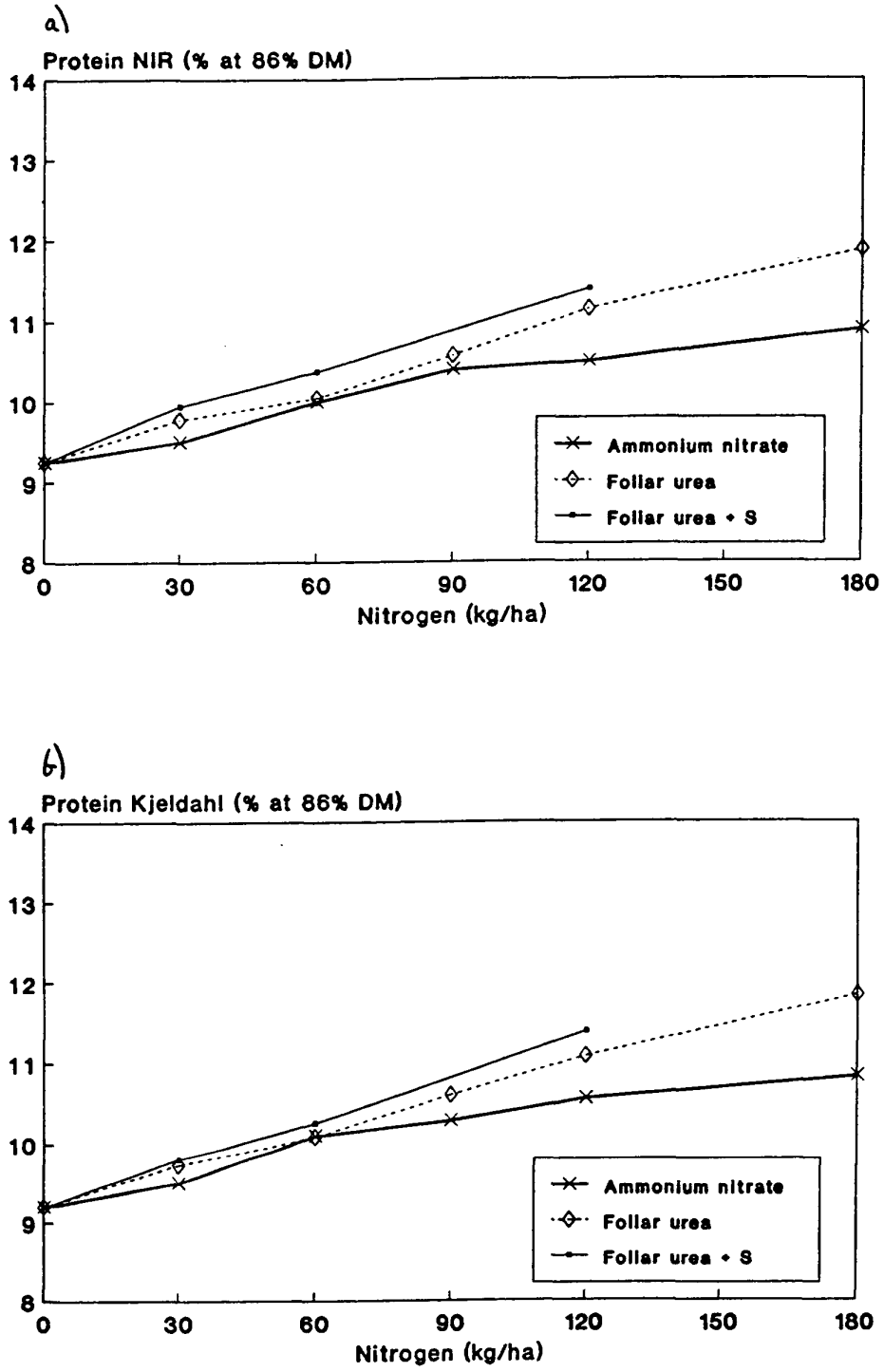


Figure 15. Comparison of flour protein to extra N according to method of analysis (Roundway 1990):

a) Analysis by NIR

b) Analysis by Kjeldahl.

Flour protein content was measured by two different procedures at FMBRA. Firstly, by the traditional Kjeldahl method which measures the total nitrogen content of the flour in all forms, and uses an established factor of 5.7 to convert N content into protein content. The second method, near infrared reflectance (NIR) spectroscopy, which measures the peptide bonds in proteins, is calibrated against flour protein content. Any differences observed between the two measurements would suggest the presence of non-protein nitrogen. Figures 15a and b show NIR and Kjeldahl data for Roundway 1990 which illustrates the average relationship found in this work. Protein measured by NIR appeared to be slightly lower than Kjeldahl measurement, and this difference was fairly consistent over the entire range of treatments. However, the difference in protein content was almost always within the error associated with the two measurements ie Kjeldahl +/-0.2% and NIR +/- 0.1% (Anon, 1991).

6.1.3 Breadmaking quality

Breadmaking quality was assessed using a combination of loaf volume and a quality score based on the loaf volume, appearance, texture, crumb structure and crumb resilience. Theoretically, it is possible for a loaf to score between 0 and 50 using this assessment system. Bread of satisfactory commercial quality should achieve a combination of high volume, above 1400ml in this process, and a loaf score in excess of 30. Baking by the LFP was used throughout the study, but in the final year some additional baking tests were carried out using the Chorleywood Bread Process (CBP).

Observed effects of extra N on loaf volume and loaf score ($p<0.05$) were as follows at the 8 sites examined:

a)	No effect from either form of extra N	0 sites
b)	Increase in loaf volume and loaf score due to increasing N applications.	8 sites
c)	High levels of foliar urea more effective than ammonium nitrate.	8 sites
d)	No effect of sulphur.	8 sites

Increasing rates of extra N always produced an increase in LFP loaf volume and overall loaf score, but the magnitude of such improvements were not always as large as might have been expected from the increase in flour protein content. Table 14 illustrates the response in LFP loaf volume (ml) induced by selected rates of each form of nitrogen fertiliser.

Table 14. Effect of extra N treatment on LFP loaf volume (mls). The corresponding flour protein content (% at 86% DM) is shown in brackets.

Site	Extra N 0 kg/ha Control	60 kg/ha Am.nit.	Urea	180 kg/ha Am.nit.	Urea
1988					
Kneesall	1235	1326	1412	1392	1450
	(7.8)	(9.1)	(9.9)	(10.2)	(11.3)
1989					
Boyt	1348	1383	1398	1413	1485
	(9.7)	(9.9)	(10.1)	(10.6)	(11.1)
Folkingham	1228	1263	1320	1325	1353
	(9.3)	(10.0)	(10.2)	(10.9)	(11.6)
Hargrave	1146	1200	1253	1273	1303
	(8.5)	(9.8)	(10.2)	(10.8)	(11.6)
1990					
Frampton	1084	1175	1147	1192	1214
	(8.3)	(9.5)	(9.5)	(10.3)	(10.9)
Roundway	1131	1154	1155	1181	1211
	(9.2)	(10.1)	(10.1)	(10.8)	(11.8)
1991					
Bishops	1534	1555	1571	1623	1774
Canning	(10.5)	(11.1)	(11.7)	(11.7)	(14.1)
Milton	1493	1511	1534	1527	1617
Ernest	(10.5)	(11.6)	(12.1)	(11.6)	(14.4)

Table 14 and the individual site data in Appendix 16 shows that, at all sites, 180 kg/ha of foliar urea-N produced significantly higher flour protein contents than from an equivalent rate of

ammonium nitrate. Comparing foliar urea and ammonium nitrate at the 60 kg/ha N rate, protein content was significantly higher from foliar urea at 4 out of the 8 sites. Similarly, at all sites LFP volume responded positively to extra N in whatever form. In 15 out of the 16 possible comparisons made in Table 4, foliar urea treatments produced higher loaf volumes than the comparable ammonium nitrate treatment - this effect was significant for 9 comparisons. Comparing the chosen treatment with the control over the 8 sites, ammonium nitrate at 60 kg/ha N resulted in 3, and at the 180 kg/ha N rate in 6 significant increases in loaf volume. The equivalent figures are 4 and 8 respectively for 60 and 180 kg/ha N as foliar urea.

Thus, foliar urea was more effective in increasing both protein content and LFP loaf volume - the difference was more significant at the 180 kg/ha than at 60 kg/ha N rate, which is closer to commercially used rates of extra N.

Table 14 also illustrates that substantial differences exist in the responsiveness of LFP loaf volume to N treatment and basic quality. For example, at the 1990 sites LFP loaf volumes were low and did not respond well to extra N. For these sites, the relationship between protein content and loaf volume was poor. In contrast, the highest loaf volumes were obtained from grain harvested in 1991 where protein contents were also high. Since Mercia was grown at all these sites, differences cannot be assigned to any interaction of variety and baking process, but must be a function of differences in the quality of flour protein between these seasons or sites.

Typical responses of LFP loaf volume to extra N are shown in Figure 16. Figure 16a shows a site where there was a good response to extra N (Kneesall, 1988) and Figure 16b shows a less responsive site (Roundway, 1990). Both sites gave higher grain protein contents from the use of extra N, permitting samples to reach the protein specifications for mill intake. In both cases loaf volume also increased with increasing rate of extra N. However, the size of the loaf volume increase was quite different and not affected by the form of N used.

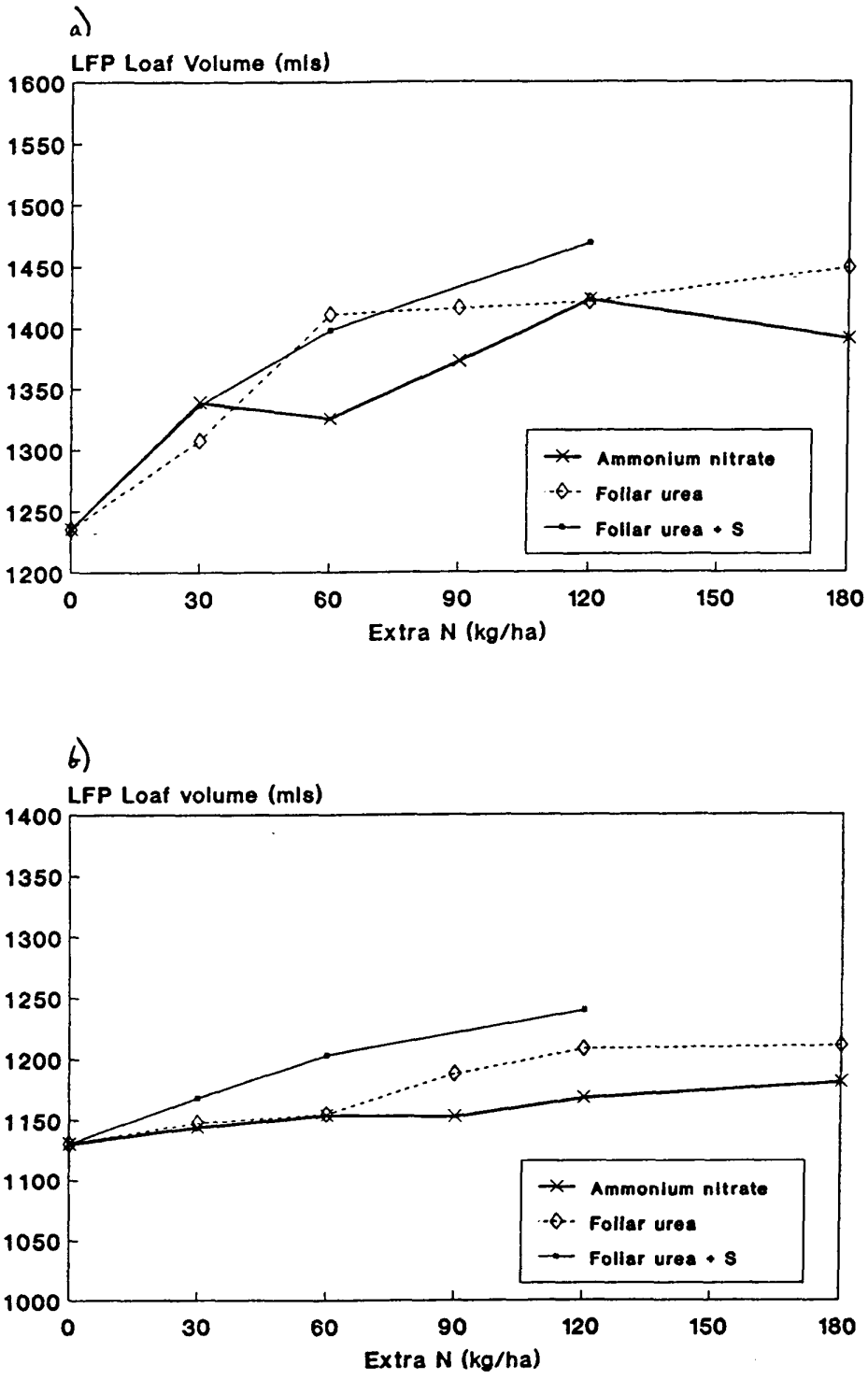


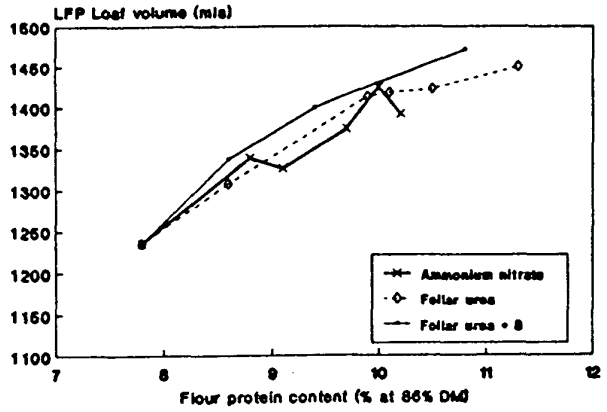
Figure 16. Variations in response of LFP loaf volume to extra N:
a) Responsive site (Kneesall 1988)
b) Low response site (Roundway 1990)

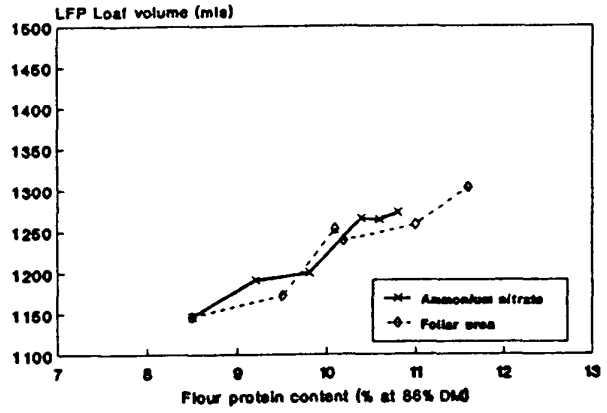
At the Kneesall site, approximately 63ml increase in loaf volume could be achieved for every 1% increase in flour protein content induced by extra N whereas at Roundway only a 31ml improvement was possible. The Roundway site produced wheat of low specific weight (69.7 kg/hl for control treatment) which resulted in below average flour extraction, but produced flour of good colour. In addition, there were suggestions that the ratio of nitrogen:sulphur in the grain was not optimal at 18.5 (see Appendix 15a), and this situation was not improved when the crop was treated with a sulphur fertiliser. The quality of the protein appeared to be satisfactory as far as the SDS sedimentation test was concerned but unsatisfactory in terms of loaf volume and protein biochemistry.

The above data illustrate that the relationship between protein content and baking quality is not a perfect one. Despite this, it is useful to examine the relationship of LFP loaf volume against flour protein content - see Figure 17 for a) Kneesall, b) Hargrave and c) Roundway representing examples of high, average and low breadmaking response sites respectively. At each site, there was a good response in flour protein content to added fertiliser N, but a marked difference in the effectiveness of this protein with regard to loaf volume.

Despite the differences in responsiveness, the general shape of the response curves for these sites is very similar. On the assumption that the relationship between protein content and loaf volume (LFP) is approximately linear, regression lines have been fitted for each site where breadmaking quality was examined in the work. Table 15 shows the relevant slopes for each regression equation. The standard error associated with each slope is given.

Exact figures should be used with some caution since the errors associated with these regressions for some sites are quite large. There were no significant differences between values for ammonium nitrate and foliar urea. It can be concluded therefore, that the flour protein produced by these forms of N is equally effective in increasing in loaf volume. It can be seen that the lines of best fit for foliar urea and ammonium nitrate can virtually be superimposed. A typical example is shown for Kneesall 1988 (Figure 17a).





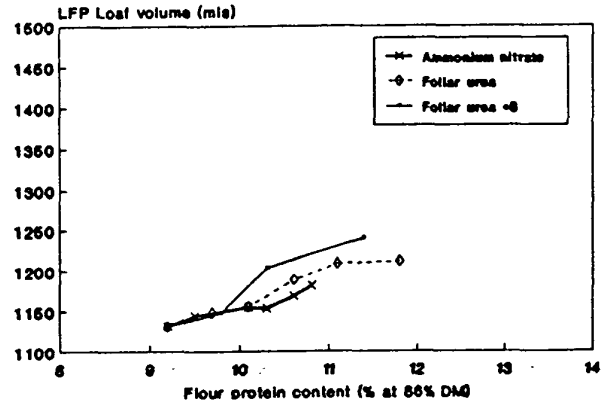


Figure 17. Variations in the response of loaf volume according to increases in flour protein.
a) Kneesall 1988
b) Hargrave 1989
c) Roundway 1990

Table 15. Response rate of LFP loaf volume to increases in flour protein content (ml per % protein) according to the form of extra N. SE's are given in brackets.

	Response rate	
	Ammonium nitrate	Foliar urea
1988		
Kneesall	70.4 (+/-10.8)	63.4 (+/- 7.7)
1989		
Boyt	71.2 (+/-12.7)	90.7 (+/-14.2)
Folkingham	65.8 (+/-20.9)	46.8 (+/-11.5)
Hargrave	56.8 (+/- 6.2)	51.6 (+/- 7.5)
1990		
Frampton	59.1 (+/-12.6)	77.8 (+/- 9.5)
Roundway	27.1 (+/- 3.9)	33.9 (+/- 4.4)
1991		
Bishops Canning	67.3 (+/-17.4)	61.9 (+/-10.3)
Milton Ernest	49.5 (+/-21.2)	44.0 (+/-10.3)

For Kneesall and Roundway, the response to extra N as foliar urea appeared to flatten at the highest rates of N, indicating a reduced effect on loaf volume improvement. Averaged over all sites, a 1% increase in flour protein content resulted in an increase in LFP loaf volume of 55ml from extra N applied as ammonium nitrate and 65ml when applied as foliar urea (comparison of control and 180 kg/ha rates of extra N).

Effects of extra N on loaf score tended to mirror the pattern observed for loaf volume. Thus, there was a positive response in loaf score to extra N at all sites and foliar urea generally produced a higher loaf score than an equivalent rate as ammonium nitrate. This is to be expected since, in addition to making a direct contribution to loaf score, loaf volume has a significant effect on some of the other baking quality parameters included in the overall score. Increases in loaf volume also tended to result in increases in crumb structure, texture and resilience scores.

Figure 18 shows the typical effect of flour protein content on loaf score. At this site there was a good response of loaf score in line with observed effects on loaf volume resulting from increases in protein content.

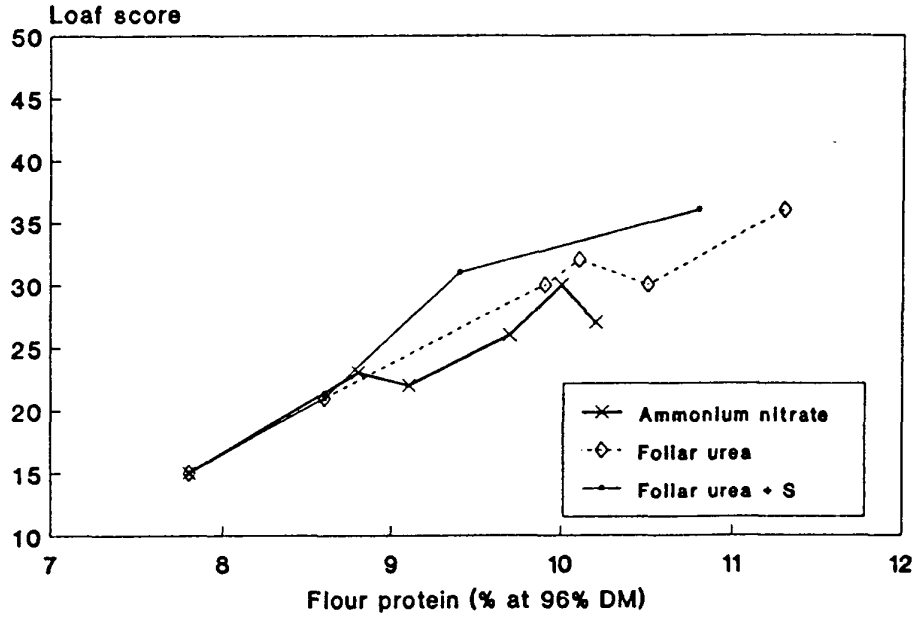


Figure 18. Effect of extra N on loaf score (Kneesall 1988)

Loaf volume and score data, together with regressions of loaf volume against protein content, suggest that there is no difference in the effectiveness of foliar urea and ammonium nitrate as far as breadmaking quality is concerned. Foliar urea has an advantage in that its application induces more flour protein than an equivalent ammonium nitrate treatment and it is therefore able to continue producing loaf volume enhancement.

Sulphur application had no significant effect at any of the sites under investigation. In the majority of trials studied, the application of sulphur produced slightly higher breadmaking performance when compared with the use of foliar urea alone but this effect did not reach significance at any individual site.

At the inception of the project it was decided to centre on the LFP baking test to assess the usefulness of protein increases induced by nitrogen treatments. However, during the timescale of the project the LFP baking test has virtually been superceded. As a consequence, the testing protocol was altered in 1991 to include the CBP as a second test baking assessment. Thus it was

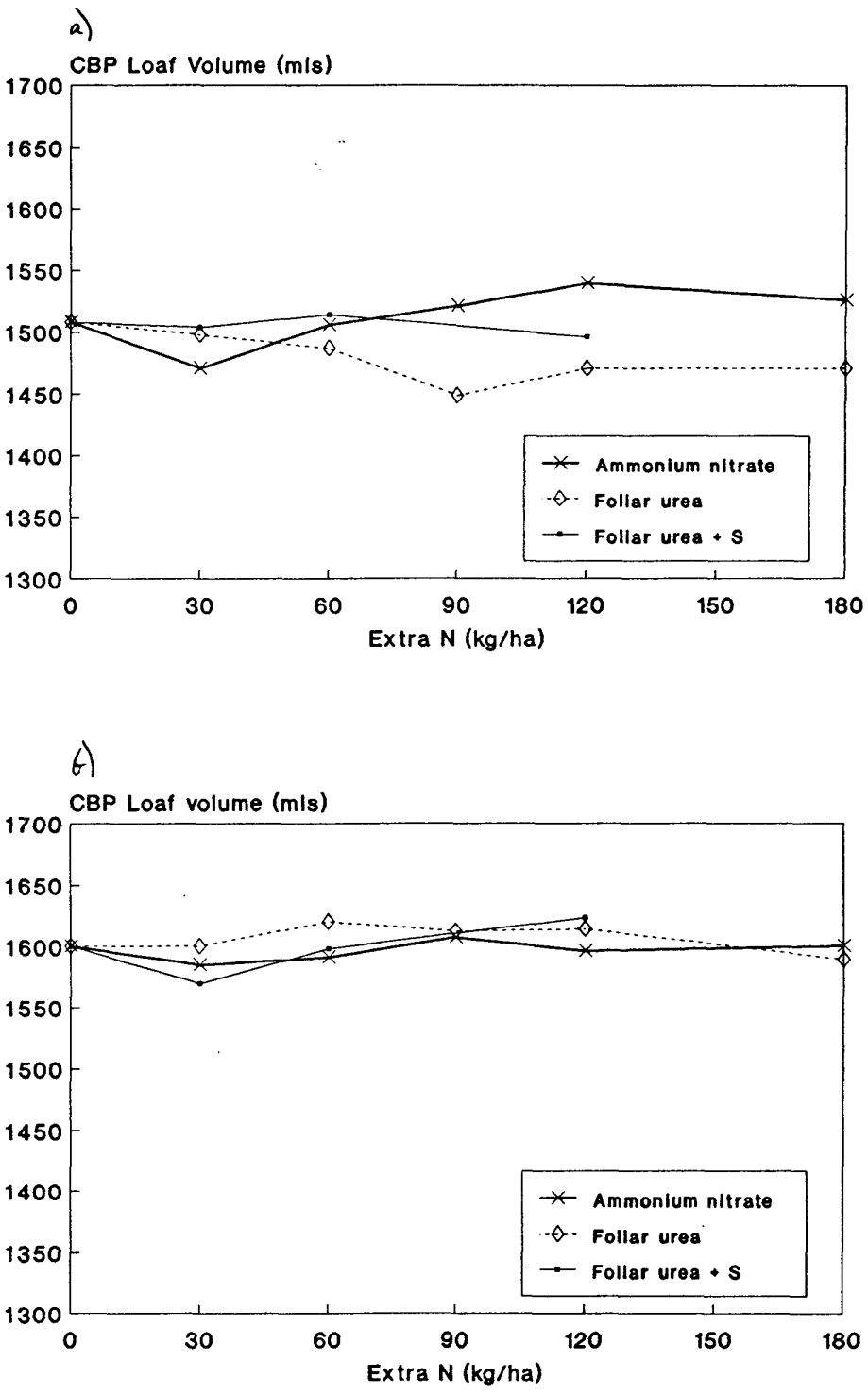


Figure 19. Effect of extra N on CBP loaf volume.
a) Bishops Canning 1991
b) Milton Ernest 1991

possible, for 1991 sites only, to compare the effects of nitrogen fertiliser treatments on breadmaking performance using the two very different baking processes. At the Milton Ernest and Bishops Canning sites, two replicates of each treatment were baked by both CBP and LFP methods.

Figure 19 shows effects of extra N on CBP loaf volume for Bishops Canning 1991 and Milton Ernest 1991. Mercia was grown at both sites. Where no extra N was used, a CBP loaf volume of 1500 ml was achieved at Bishops Canning, and 1600 ml at Milton Ernest. It is clear from Figure 19 that there was no significant response of CBP loaf volume to extra N in either form. The trials examined were characterised as having high grain protein contents - the control grain protein contents were 11.9% for Bishops Canning and 12.3% for Milton Ernest respectively. Both sites, therefore, produced wheat which would be acceptable at mill intake without extra N treatment. It should be noted that all Trial Series A sites produced high grain protein contents in 1991 and that the sites selected for milling and baking assessment at FMBRA were two of the lower protein sites.

Furthermore, the variety Mercia was classified as 6 for quality on the 1993 NIAB Recommended List (Anon, 1993). This means that the variety is capable of producing CBP bread of satisfactory, but not the highest quality. Loaf volumes of above 1500 ml (400 g bread) are considered to be good and above 1600 ml to be exceptional for this variety. At the sites under consideration therefore, it is possible that further improvement in loaf volume would be unlikely regardless of any increases in protein content.

At the Bishops Canning site, there was considerable variability in CBP loaf volume results (LSD = 58 ml), and there were only two comparisons where ammonium nitrate treatment gave a higher loaf volume than foliar urea treatment. However, the picture is complicated at this site since the addition of extra nitrogen also resulted in rather low specific weights, and unacceptably high flour colour values. The latter can reduce loaf volume. This suggests that some of the protein content may have been locked into the bran portion of the grain, which would have resulted in an increase in flour colour, but is non-functional in terms of breadmaking. Foliar urea treatments consistently produced higher flour colour values than the equivalent ammonium nitrate treatment

and, therefore, foliar urea treated samples may have been more severely affected in respect to the presence of non-functional protein at this site.

At the Milton Ernest site there were no significant differences in CBP loaf volume: control treatment 1600 ml, maximum 1623 ml, LSD (5%) = 44 ml.

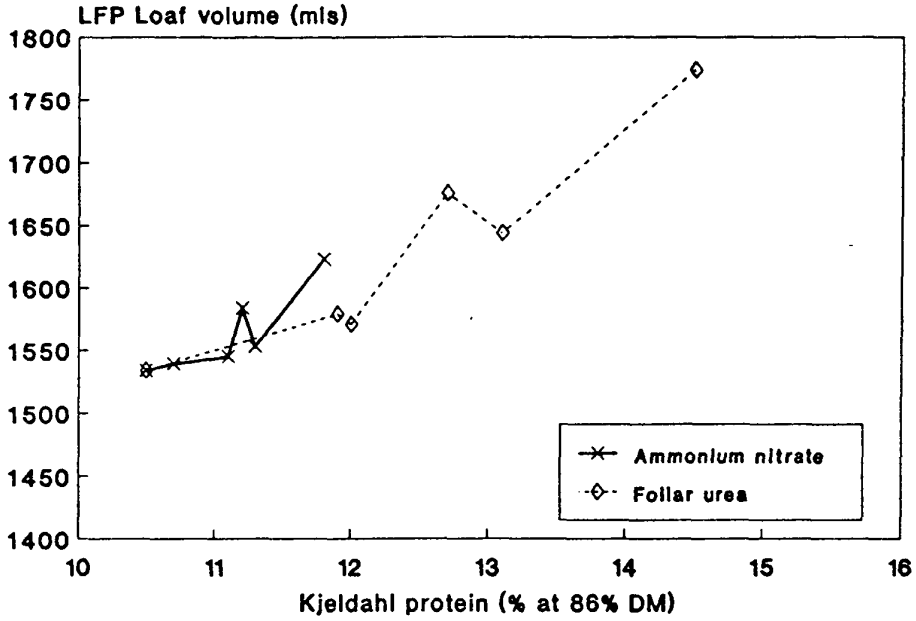
Direct comparison of the effects of increasing flour protein content on LFP and CBP loaf volume for these sites are shown in Figures 20a and b, and Figures 21a and b. These illustrate quite clearly the differences in effectiveness of added protein in terms of increasing loaf volume for the two baking processes used.

Regression analysis of loaf volume (LFP and CBP) against protein content for these two sites assumes that a straight line relationship exists between these parameters. Table 16 shows the relevant slopes for each regression equation. In addition, the standard error associated with each slope is presented as the +/- value in order to assess statistical significance of differences.

Table 16. Response rate of LFP and CBP loaf volume to increases in flour protein content (ml per % protein) according to the form of extra N. SE's are given in brackets.

	Response rate of LFP loaf volume	
	Ammonium nitrate	Foliar urea
Bishops Canning	67.3 (+/-17.4)	61.9 (+/-10.3)
Milton Ernest	49.5 (+/-21.2)	44.0 (+/-10.3)
	Response rate of CBP loaf volume	
	Ammonium nitrate	Foliar urea
Bishops Canning	33.5 (+/-20.2)	-4.7 (+/- 7.7)
Milton Ernest	4.6 (+/ -7.2)	-1.1 (+/- 4.2)

The errors associated with these regressions were often large and no significant differences were found. However, it is clear that the response in loaf volume due to flour protein was quite different depending on whether the LFP or CBP baking process was used.



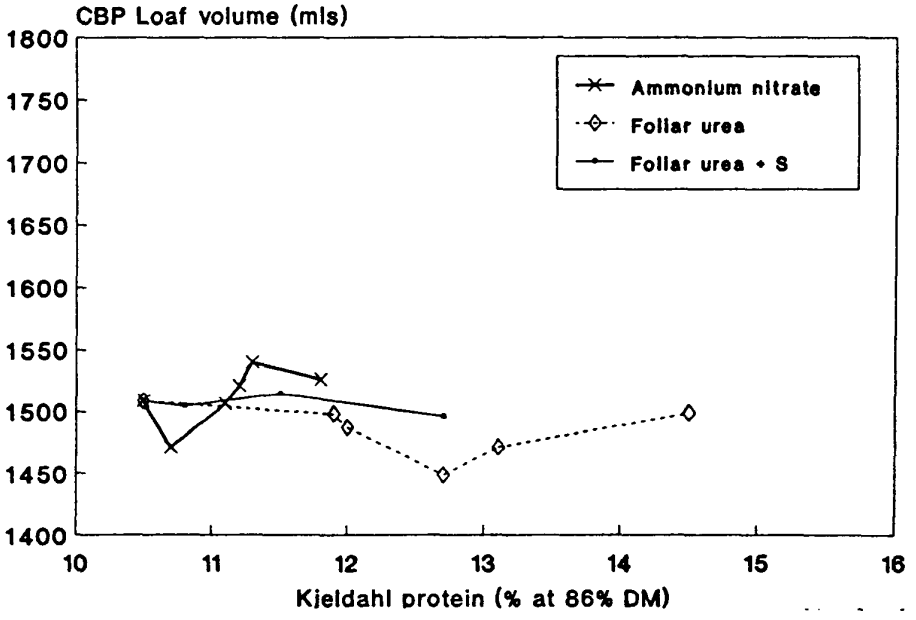


Figure 20. Comparison of LFP and CBP baking methods and the response of loaf volume to flour protein content (Bishops Canning 1991):
a) Response of LFP loaf volume.
b) Response of CBP loaf volume.

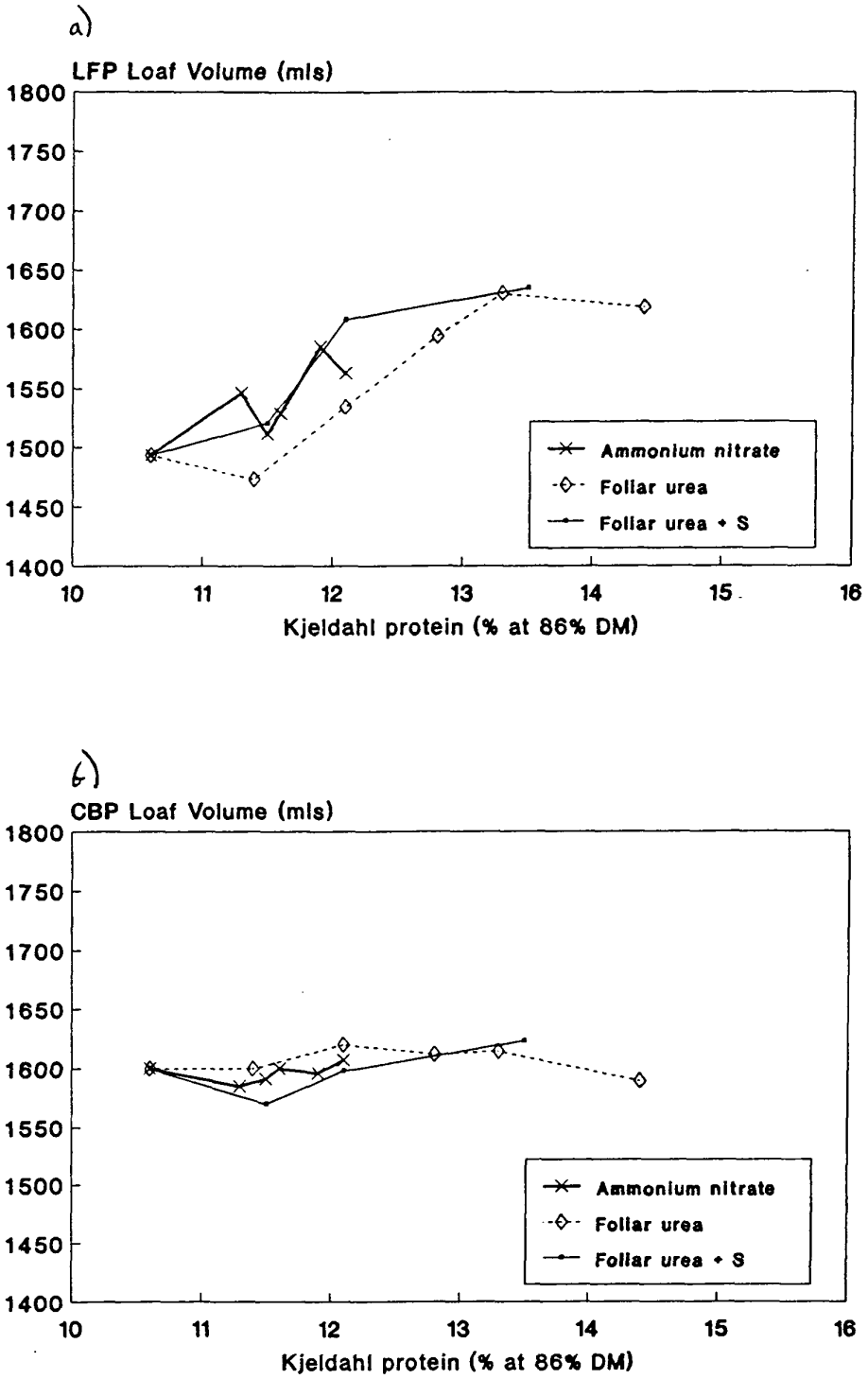


Figure 21. Comparison of LFP and CBP baking methods and the response of loaf volume to flour protein content (Milton Ernest 1991):
a) Response of LFP loaf volume.
b) Response of CBP loaf volume.

At both sites, there was a good response in LFP loaf volume to increases in protein content due to foliar urea treatment (see Figures 20a and 21a). Such responses are consistent with effects observed for other sites examined in other seasons. It is only at the highest flour protein content at Milton Ernest that the curve appears to plateau. An average increase of 37 and 60 ml was produced for every 1% increase in protein content induced by foliar urea at Milton Ernest and Bishops Canning respectively.

At Bishops Canning, there was a similar response in LFP loaf volume to both forms of extra N, the size of the response being limited by the flour protein content. At Milton Ernest, the highest rates of ammonium nitrate produced no further increases in protein content and therefore gave a similar response in LFP loaf volume. Significant increases in LFP loaf volume occurred at the 120 kg N/ha level of application only.

For the CBP baking test there was no loaf volume response to increases in protein content induced by foliar urea or ammonium nitrate at the Milton Ernest site. At Bishops Canning there was a positive trend (NS) towards increasing loaf volume with increasing protein content (see Figures 20b and 21a).

It is unfortunate that this comparison of baking methods was only carried out in samples from the 1991 harvest, which were of high protein content, but lacking in other quality respects. The two baking methods employed are quite dissimilar in terms of recipe and processing details which has resulted in very different responses to the protein increases generated by either fertiliser treatment. Attempts have been made in the discussion section to explain the differences observed in baking performance between LFP and CBP for the above sites.

6.1.4 **Gluten Quality Assessment**

When flour and water are mixed, the proteins in the flour hydrate to form a cohesive gluten network which, in a good breadmaking wheat variety, is capable of retaining the gas during breadmaking. In the Glutomatic apparatus, flour and buffered salt solution are mixed to form a

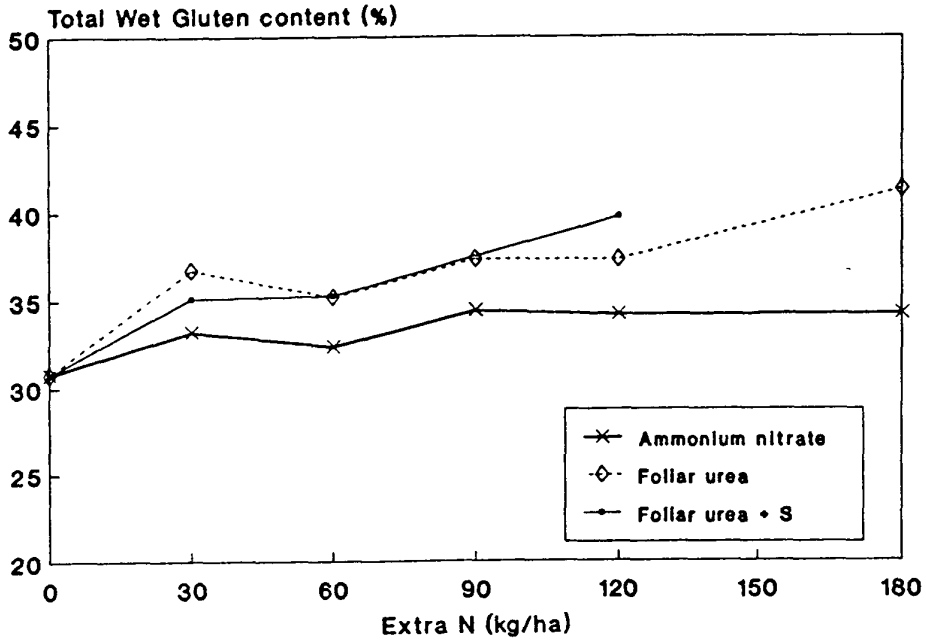


Figure 22. Effect of extra N on wet gluten content (Milton Ernest 1991)

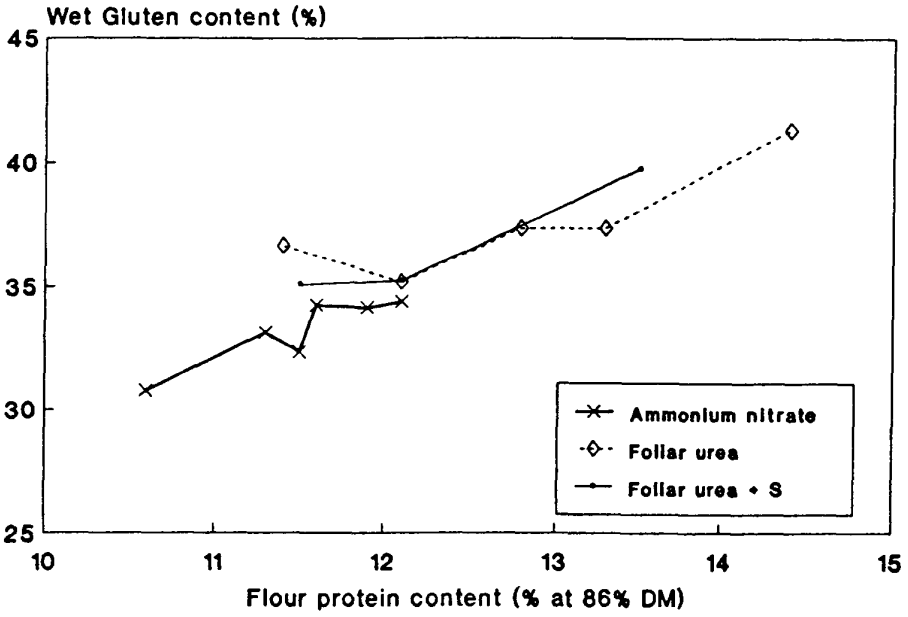


Figure 23. Effect of flour protein content on wet gluten content (Milton Ernest 1991).

dough. The starch and water-soluble protein are then progressively removed by a gentle mixing and washing action to leave behind the insoluble gluten proteins. The prepared gluten is centrifuged at 6000 rpm to remove excess water. During this centrifugation process some of the gluten is forced through the pores of a special sieve. The amount of gluten on each side of the sieve is then measured; the total amount provides a value for the wet gluten content; the ratio of material retained on the sieve divided by the total gluten weight x 100 is termed the Gluten Index. This Index is used to provide a measure of the quality of the gluten. High values should indicate strong gluten with breadmaking potential, low values should indicate gluten weakness.

Gluten measurements were only carried out at Milton Ernest 1991. Analysis of the results showed that there was a gradual increase in the wet gluten content with increasing rate of extra N. Thus, as protein content increased, so the quantity of wet gluten, measured by the Glutomatic, also increased in line with expectations (Figure 22). This effect just failed to reach statistical significance when extra N was applied as ammonium nitrate, but was significant for all rates of foliar urea. In addition, the ratio of wet gluten content to flour protein content remained relatively constant regardless of N treatment, with the % wet gluten content being consistently around 2.9 times the measured flour protein content.

Figure 22 shows the relationship between wet gluten content and flour protein content. This suggests that the increased protein content induced by both forms of extra N, produces insoluble gluten protein which would be expected to improve baking performance.

The effects of extra N on Gluten Index at the Milton Ernest site were also examined (results in given in Appendix 16). Gluten Index values were relatively high indicating a good quality, strong gluten typical of a variety like Mercia, but they showed a high level of variation (CV 14.3%). Against this background, Gluten Index values tended to decrease with increasing levels of ammonium nitrate suggesting reduced gluten strength. This effect only reached statistical significance at the highest N rates. There was no significant response in gluten characteristics as a result of added foliar urea, but again the trend was towards a decrease in Gluten Index as urea-N rates increased.

Thus foliar urea treatment appeared to produce more gluten of similar quality, and extra N as ammonium nitrate resulted in more gluten of apparently poorer quality. However, these comments are based on data from 1 trial site and it would be dangerous to draw firm conclusions from this limited information. The Gluten Index test is also unproven in relation to assessment of gluten quality in UK wheats (Bhandari, pers.comm).

Biochemical studies (see section 7) have included gel-protein measurements and protein fractionation which provides information that supports the conclusion that protein of satisfactory quality is produced from normal commercial rates of applications of late foliar urea - ie 30-40 kg/ha N.

6.2 Trial Series B. Effect of timing of foliar urea application

In order to influence breadmaking performance in a positive way, firstly nitrogen fertiliser must be adequately absorbed by the plant and secondly it must be converted into protein, particularly the large glutenin type proteins essential for breadmaking. Correct timing of such applications is important in order to maximise the potential quality improvement and to allow sufficient time for metabolic processes in the plant and conversion to storage proteins.

The following comparisons have been made to assess the effect of different timings of foliar urea and sulphur applications. In all cases the effect of time of application of foliar urea has been compared with the control treatment. In order to assess any effects of sulphur application on test variables, the foliar urea+sulphur treatment has been compared with the equivalent foliar urea only application.

6.2.1 Milling quality

A total of 9 timing trial sites were examined. Significant effects ($p<0.05$) on milling quality parameters can be summarised as follows:

A. Flour extraction rate

a)	No effect of foliar urea application	7 sites
b)	Increase from one or more timing	1 site
c)	Decrease from one or more timing	1 site
d)	No effect of sulphur treatment	9 sites

B. Flour colour grade

a)	No effect of foliar urea application	4 sites
b)	Increase from one or more timing	5 sites
c)	Decrease from one or more timing	7 sites
c)	No effect of sulphur	9 sites

Results show that timing of foliar urea application had virtually no effect on performance in the milling process. As in Trial Series A, season and variety had a greater effect on flour extraction rate than any treatment. Only 2 significant treatment effects were recorded. Figure 24 shows the

situation at Bethersden 1989, where both timing and sulphur applications produced small, but significant, effects on this parameter. Since only small isolated effects were observed in Trial Series A, where up to 180 kg/ha extra N was applied, it is not surprising that the use of only 30-40 kg/ha of foliar urea-N in Trial Series B had no significant effect on flour extraction.

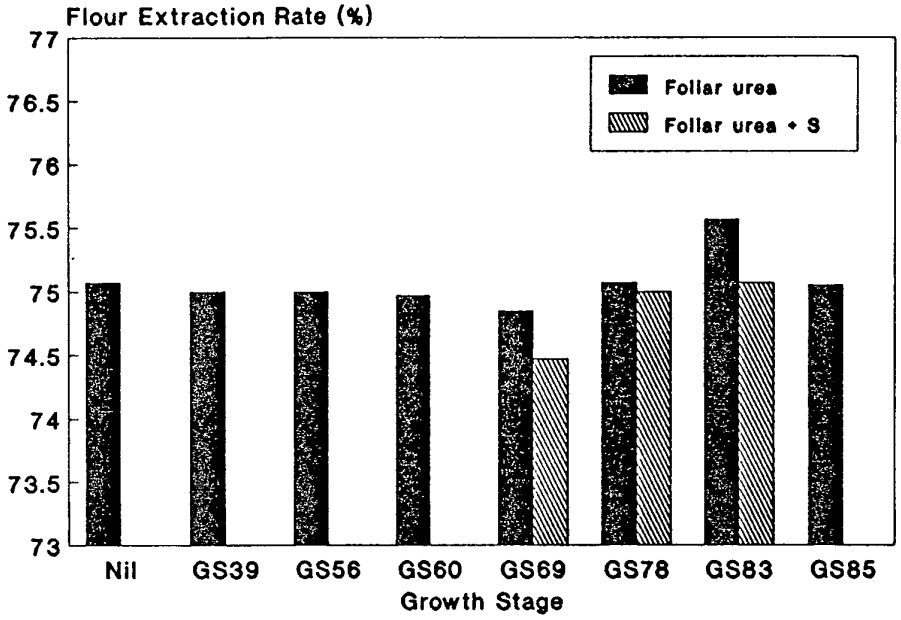


Figure 24. Effect of foliar urea application timing on the extraction rate of white flour (Bethersden 1989).

The timing of urea applications did affect flour colour grade. Results indicated that the application of 30-40 kg/ha of extra N as foliar urea produced an average increase of 0.4 GCF units, though such effects were not serious given the generally good flour colour grade values observed. Significant increases ($p<0.05$) were recorded at 5 out of 9 sites tested. Grade colour figures were less than 1.0 GCF units and negative at some trials (values of less than 2.5 GCF are required for most white breadmaking flours). Foliar urea applied at or after GS83 generally gave flour with similar GCF values to control treatments. Occassionally applications at around GS70 produced the same effect: significant ($p<0.05$) reductions in GCF were observed at 7 out of 9 sites tested.

Thus, as far as milling quality is concerned timing of foliar urea applications were relatively unimportant. For this reason, the optimum growth stages for application have not been considered.

6.2.2 Flour protein content

Since protein losses on milling were not found to be seriously affected by applications of up to 180 kg/ha N as foliar urea, the levels of treatment used in Trial Series B were not expected to have any effect.

However, there has been a suggestion that very late urea applications may not be fully incorporated into useful proteins for breadmaking purposes. As in Trial Series A, flour protein content was measured by traditional Kjeldahl and NIR methods. Any differences observed between the two measurements would suggest the presence of non-protein nitrogen. Comparing these two flour protein values, for each time of application shows that there was generally a consistent difference between Kjeldahl and NIR results. Kjeldahl results were, on average, 0.2% higher than NIR values but there was no evidence that this difference was influenced by timing of N treatment. Similar differences were observed in Trial Series A. This suggests that, if foliar urea-N is taken up into the grain, then it is converted into some form of protein.

The application of 30-40 kg/ha N as foliar urea consistently produced a small increase in flour protein content across all nine sites examined. The mean maximum response in flour protein content obtained from this extra N was 0.66%, ranging from 0.32-0.96%. However, it should be remembered that, as with Trial Series A, sites were selected partly on the basis of protein response to foliar urea (see Section 5.2.2 for full picture across all sites).

Significant responses ($p< 0.05$) in flour protein content were observed as follows:

a)	No effect of foliar urea	0 sites
b)	Increase from one or more timing	9 sites
c)	Decrease from one or more timing	9 sites
d)	No effect of sulphur	9 sites

For all sites examined, foliar urea application produced increases in flour protein content, but equally in all trials the timing of such treatments was important. Figure 25 shows the typical effect of different timings of foliar urea on flour protein content (Morley 1989). The application of 40 kg/ha N at this site produced a positive response in flour protein content from foliar urea applied up to GS75. Beyond this point flour protein content declined. This suggests that, for this

site, uptake of N into the grain was less efficient from applications after GS75, and by GS87 had stopped altogether - the flour protein content of the GS87 treatment was the same as for the control treatment.

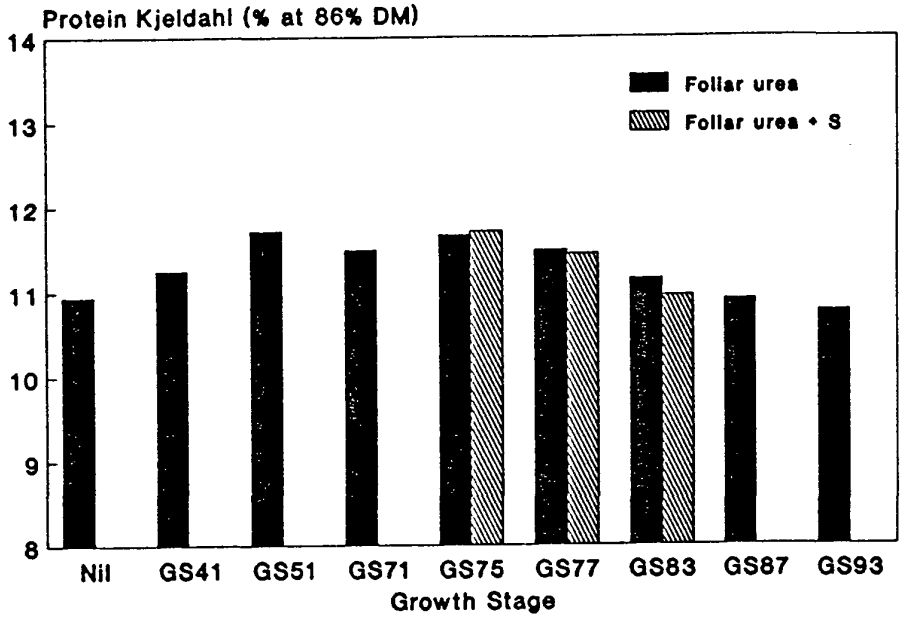


Figure 25. Effect of foliar urea application timing on flour Kjeldahl protein (Morley 1989).

The critical growth stages varied from site-to-site and season-to-season. However, for the sites examined, the maximum response in flour protein content could normally be achieved when foliar urea was applied at or before GS75. In 5 out of the 9 trials, a foliar urea application after GS83 failed to produce a significant improvement in flour protein content when compared with the control. At 1 site (Detling 1988), foliar urea applied at GS69 failed to give any significant increase in flour protein content.

Using the general growth stage limits estimated in the agronomic section of this project (Table 8) and with reference to individual trial results (Appendix 17), it is obvious that effects on flour protein content are consistent with grain protein results and the optimum time of application is generally around the milky ripe stage of grain development (GS70-79). Of more importance, is the fact that, at most sites, applications beyond this point do not have any positive effect on flour protein content.

The addition of sulphur produced no significant effect on flour protein content, although there was a trend (ns) for sulphur treatment to produce slightly higher flour protein contents.

6.2.3 Breadmaking quality

Extra N as foliar urea produced some useful increases in grain protein content. At 3 sites examined by FMBRA, 30-40 kg/ha extra N as foliar urea converted unacceptably low protein grain into samples which would be accepted at mill intake on the basis of this parameter. However, it should be remembered that the magnitude of such protein increases was relatively small at around 0.45%.

Significant effects ($p<0.05$) of timing of foliar urea application and sulphur treatments were observed in LFP loaf volume and loaf score as follows:

A LFP Loaf volume

a)	No effect of foliar urea application	3 sites
b)	Increase from one or more timing.	6 sites
c)	Decrease from one of more timing	7 sites
e)	No effect of sulphur treatment	9 sites

B Loaf score

a)	No effect of foliar urea	5 sites
b)	Increase from one or more timing.	4 sites
c)	Decrease from one or more timing	7 sites
e)	No effect of sulphur treatment	9 sites

Thus, at 6 sites, foliar urea application produced a significant improvement in measured LFP loaf volume. An average increase of 65ml was obtained from 30-40 kg/ha extra N applied as foliar urea. At most sites, normal commercial rates of foliar urea resulted in worthwhile increases in LFP loaf volume.

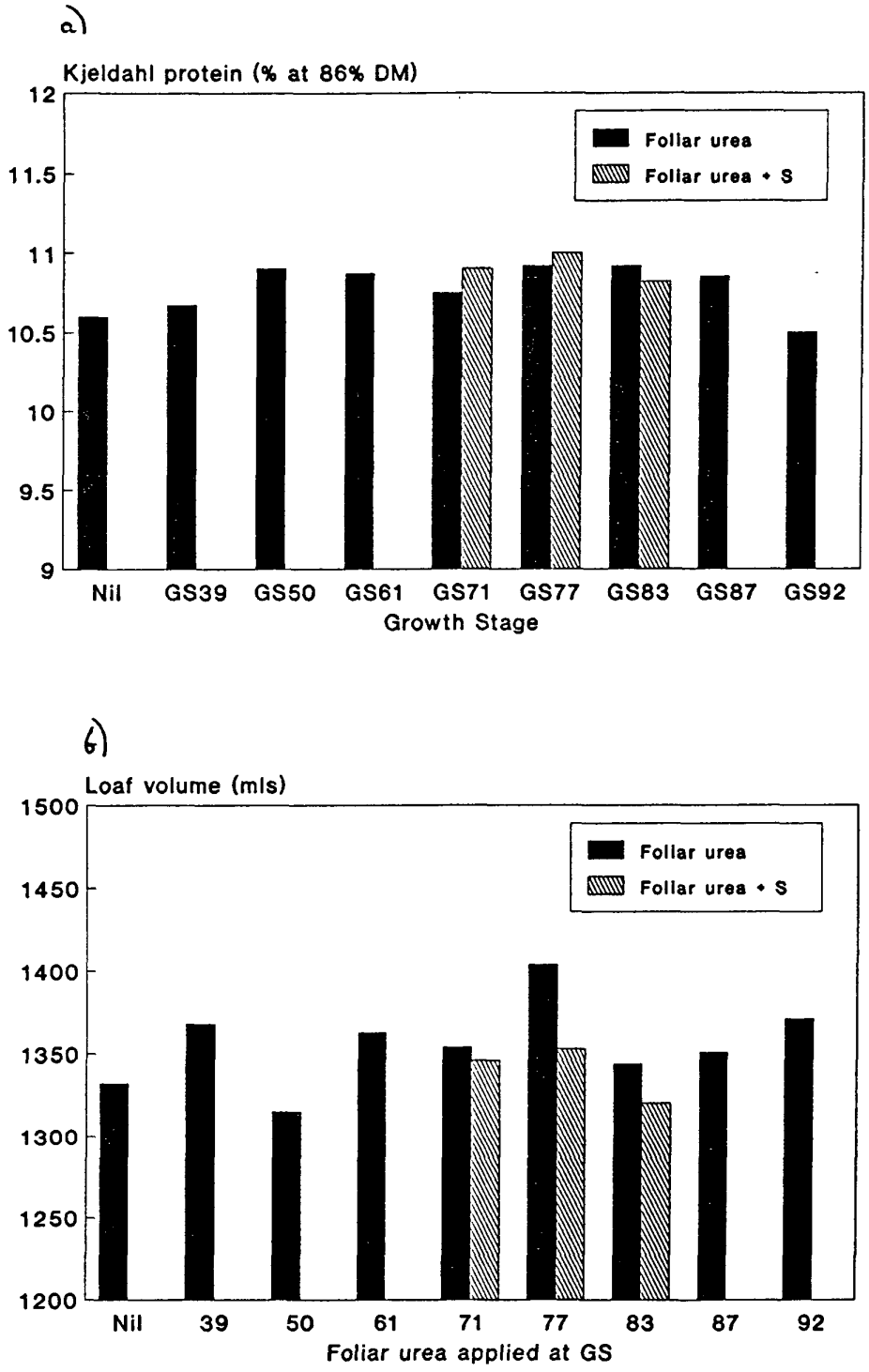


Figure 26. Effect of foliar urea application timing (Terrington 1989) on:

a) Flour Kjeldahl protein
b) Loaf volume

Figures 26a and b show typical responses of Kjeldahl protein and LFP loaf volume to different timings of application of foliar urea at Terrington 1989. The application of 30 kg/ha N at this site produced an increase in flour protein content of 0.33% at its maximum, achieved from both GS77 and GS83 applications. This increase in protein content resulted in an increase of nearly 72ml in loaf volume over the control from foliar urea applied at GS77. Beyond GS77, no further improvement in loaf volume resulted from added foliar urea. Thus, at this site the cut-off point for protein accumulation and improvement in breadmaking quality occurred almost simultaneously.

Given the limitations of the sequential growth stages used in this work (ie nominally at 10 day intervals), Table 17 shows the growth stage at which wheat first failed to produce a positive response to applied foliar urea, in terms of flour protein content and breadmaking quality. It is important to realise that the growth stages, indicated would not all produce significant reductions, but are merely the first timepoint at which a reduction in the above parameters was noted.

Table 17. Growth stages at which flour protein content and loaf volume first start to decline.

	Protein content	Loaf volume
1988		
Detling	69	67
1989		
Bethersden	78	78
Morley	87	83
Terrington	87	83
1990		
Bethersden	83	75
Sutterby	75	85
Terrington	75	77
1991		
Boxworth	75	75
Milton Ernest	83	85

Table 17 illustrates that the cut-off points controlling the effectiveness of foliar urea applications, as regards accumulation of protein and improvement in breadmaking performance, are reasonable well synchronised. With the exception of Sutterby 1990, the growth stages quoted are within one timepoint in the trial.

The sites which produced positive responses in breadmaking quality were not necessarily those which produced the largest protein response. However, of the sites which failed to give any significant response in terms of LFP loaf volume to foliar urea application, Sutterby had a low control flour protein content of 8.8% which extra N only managed to increase to a maximum of 9.5% (ie. below normal breadmaking flour specifications), whilst Bethersden 1989 and Detling 1988 produced bread of satisfactory quality without added nitrogen treatments.

Effects on overall loaf score were small. Significant effects were observed at 3 sites as a result of extra N as foliar urea. The following sites showed significant increases in loaf score when 30-40 kg/ha N was applied as foliar urea - Terrington 1990; Boxworth 1991; Milton Ernest 1991. For typical sites shown in Figure 27, loaf score reached a peak when foliar urea was applied at GS77 (Morley) and GS69 (Bethersden). Only small improvements were obtained when 30-40 kg/ha N was applied and, as for Trial Series A, much larger differences occurred between sites and between seasons. Treatments had no consistent effect on the appearance, crumb texture, crumb structure or resilience of the loaf.

There was no consistent or significant effect of sulphur fertiliser treatment on loaf score at any site.

6.2.4 Gluten Quality assessment

Gluten measurements were performed on samples from both Milton Ernest and Boxworth sites in 1991. As for Trial Series A, wet gluten contents generally followed changes in flour protein content - i.e. when extra N treatment resulted in an increase in protein content, percentage wet gluten content also increased. In addition the ratio of wet gluten weight to flour protein content was relatively constant at around 2.9 throughout both trials.

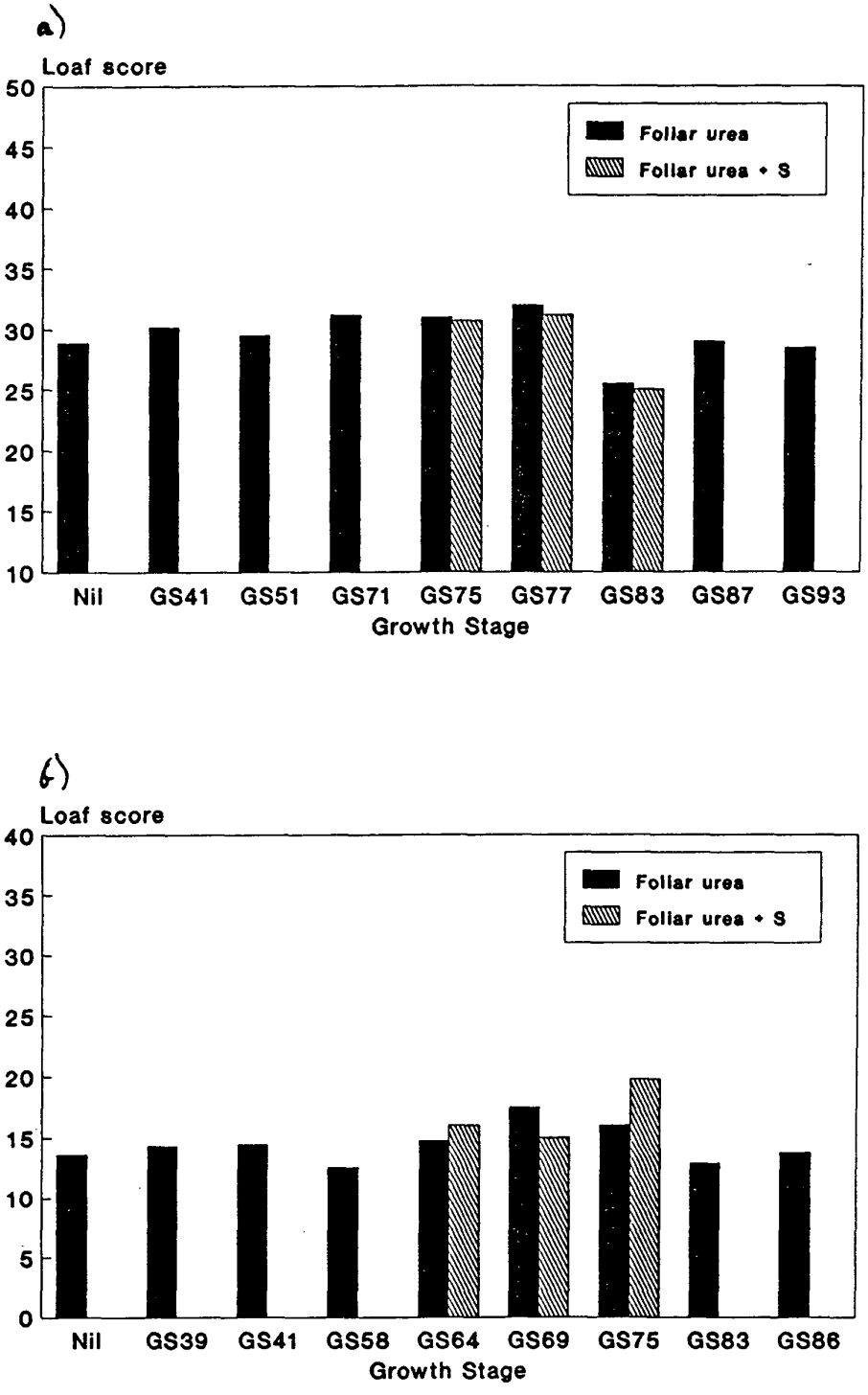


Figure 27. Effect of foliar urea application timing on loaf score:

a) Good breadmaking quality (Morley 1989)
b) Poor breadmaking quality (Bethersden 1990)

Due to the limited amount of testing for gluten quality, it is not possible to determine an accurate growth stage for maximum gluten content (see data in Appendix 17). For both sites, the amount of recoverable wet gluten reached a maximum at roughly the same time point as flour Kjeldahl protein content -ie. GS75 at Boxworth, and GS83 at Milton Ernest.

This suggests that as long as the plant is capable of taking up foliar urea it is also able to convert this N into proteins which contribute to wet gluten content. Thus, again there is no evidence that late foliar urea treatments are not completely converted into useful proteins by the wheat plant: if foliar urea can be absorbed by the plant during the later stages of development it appears that it can be converted into useful gluten proteins.

At both sites, Gluten Index values showed a high degree of variation, and were not significantly affected by any extra N treatment. Again, Gluten Index values were relatively high throughout both trials as expected for the variety Mercia. Previous experience has shown that laboratory repeatability is poor for this determination. This study has confirmed this variability, and it has not been possible to detect any convincing effects.

7. RESULTS - PROTEIN BIOCHEMISTRY

Due to the complex and costly nature of protein biochemistry determinations, there was a high degree of selectivity to identify the samples for analysis (see Table 4). The nature of determinations also varied from season to season - a summary of the analytical methods used is given in Section 3.3.

7.1 Studies of Kneesall 1988 (Trial Series A)

This site was chosen for more detailed study because the response of baking tests to extra N was the largest of those studied in the 1988 harvest year.

7.1.1 Aminoacid analysis and protein content of flours

Flour samples from all treatment replicates were examined for total aminoacid content. The effect of extra N on total aminoacids (Figure 28) for the different treatments is very similar to the relationships with Kjeldahl and NIR estimates of protein in the same samples (not shown). Both forms of extra N caused significant increases in calculated flour protein content, with foliar urea N being more effective than ammonium nitrate.

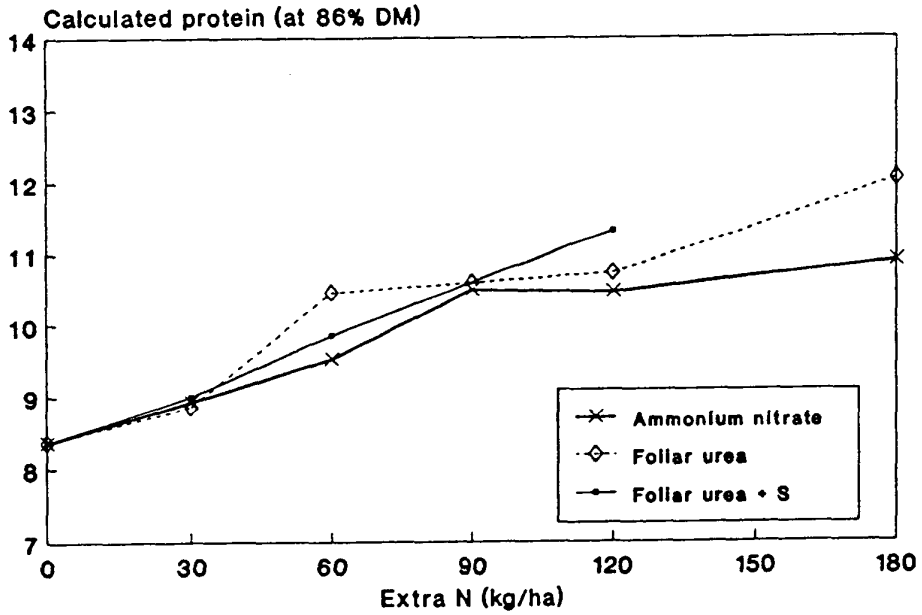


Figure 28. Effect of extra N on the calculated total protein from individual aminoacid analysis (Kneesall 1989).

There was a good linear correlation (not shown) between the protein content calculated from aminoacid and that based on Kjeldahl N analysis, though overall the values were biased upwards by about 0.5% protein, relative to the Kjeldahl values, which were in turn about 0.2% protein higher than the estimates by NIR. The data were obtained to assess the extent of any accumulation of non-aminoacid forms of N in the grain that might occur from late application of urea. Such N would in theory appear as a deficit of protein content calculated from aminoacids, relative to that calculated from the total (Kjeldahl) nitrogen.

Since, on chemical grounds, the recovery of N was very likely to be less complete as aminoacids, than as ammonia from Kjeldahl digestion, it seems that there must have been a methodological discrepancy in the estimates of protein content from aminoacid analysis. The apparent systematically greater yield of N aminoacids obviated any possibility of using this approach to detect any accumulation of non-protein N in the samples grown with high levels of late urea.

7.1.2 N as free aminoacids in flours

Flours from replicates of selected treatments were analysed for the content of "free" aminoacids, to assess if they were a significant form of non-protein N. Table 18 shows that the amounts of N in this form account for less than 1% of the total N, or less than 0.1% flour protein in a flour with 10% protein, even in samples that received the highest levels of late foliar urea. Such small inaccuracies in estimation of true protein content are not significant in the context of bread baking quality.

The results in Table 18 are also presented as the percentage of the free aminoacid N that was present in the most abundant aminoacids found. In all cases, almost half of this N was in the form of aspartic acid + asparagine, with glutamic acid + glutamine and serine as the next largest contributors. This spectrum of biochemically labile aminoacids had been changed only a little within the endosperm, even by the most extreme fertiliser treatments.

Table 18. Kneesall 1988 - N present as free aminoacids in flours

Treatment	Kjeldahl N in free form (%)	Proportion of % free aminoacid N found as: Asp+Asn	Glu+Gln	Ser
Control	0.83	48	15	9.5
	0.90	48	15	8.6
Urea	0.68	45	15	12
(180 kg N/ha)	0.73	47	16	11
Urea + S	0.73	49	13	12
120 kg N/ha)	0.72	49	15	12

7.1.3 Cysteine residues in flours

The sulphur-containing aminoacid cysteine merited special attention, because the amount present in the glutenin fraction of gluten plays a central role in the rheological properties of dough and its response to oxidising improvers. The quantity of glutenin subunits present as the crucial cysteine (disulphide)-linked macropolymers can be influenced by both the nitrogen and sulphur status.

The content of cysteine residues in the flours was significantly increased at all rates of extra N (see Figure 29). This data illustrates that extra N applied as ammonium nitrate produced significantly more flour cysteine residues than when applied as foliar urea. Addition of sulphur to the foliar urea produced a small but consistent trend for increased cysteine but this was not statistically significant.

The flours produced with ammonium nitrate generally contained less protein than those produced with foliar urea. Thus the proteins from ammonium nitrate contained more cysteine per unit protein, particularly at higher N applications (see Figure 30). The protein induced by ammonium nitrate N gives a curve that is significantly above and of greater slope than the urea-induced protein. Where sulphur was applied, there was a slightly higher cysteine enrichment than with foliar urea alone.

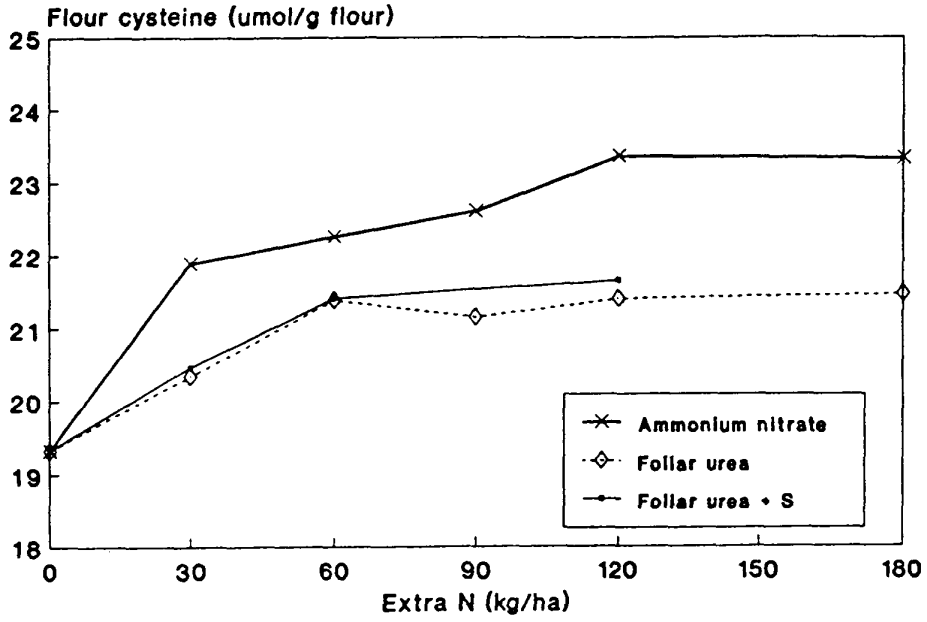


Figure 29. Effect of extra N on flour cysteine content (Kneesall 1988)

The functional significance of this enrichment in cysteine for the baking quality of flours was assessed by plotting the loaf volume against the flour cysteine content (see Figure 31). The flour samples fertilised with ammonium nitrate were clearly distinguished from those fertilised with urea, which was not the case when the loaf volumes were plotted against flour protein, in that their points lie on a curve which is both below and of lesser slope than the points of the flour samples fertilised with urea. In other words, the urea-induced cysteine is associated with a high baking performance, which may imply that it is in a more useful form, on average, than the ammonium nitrate-induced cysteine.

The analyses conducted on flour samples from Kneesall 1988 did not directly examine the location of the cysteine residues amongst the various types of flour proteins. However, the results found are consistent with the urea-induced cysteine being biased towards the functional gluten proteins, and that induced by ammonium nitrate towards non-functional albumin and globulin protein fractions.

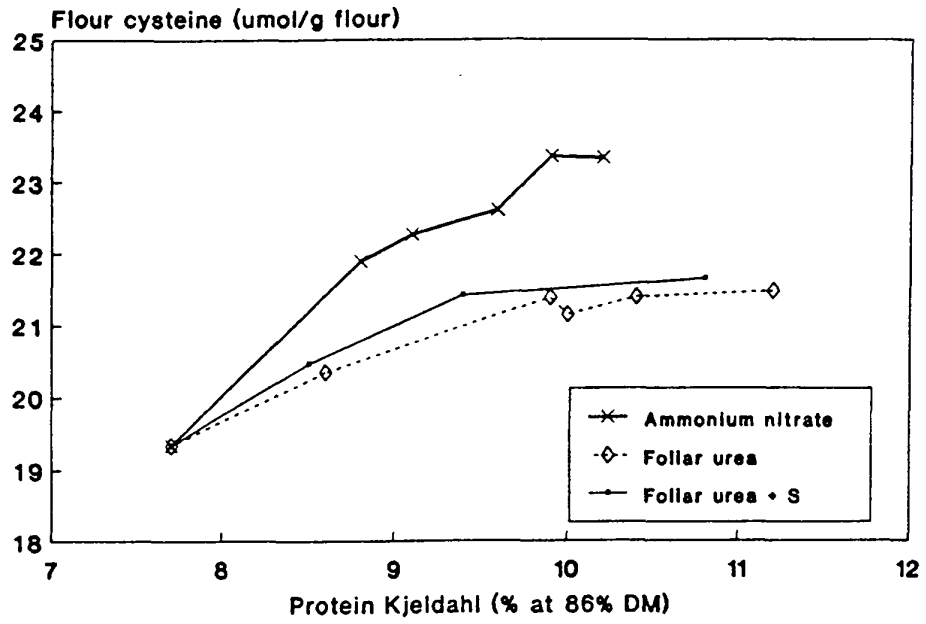


Figure 30. Effect of flour protein content on flour cysteine content (Kneesall 1988)

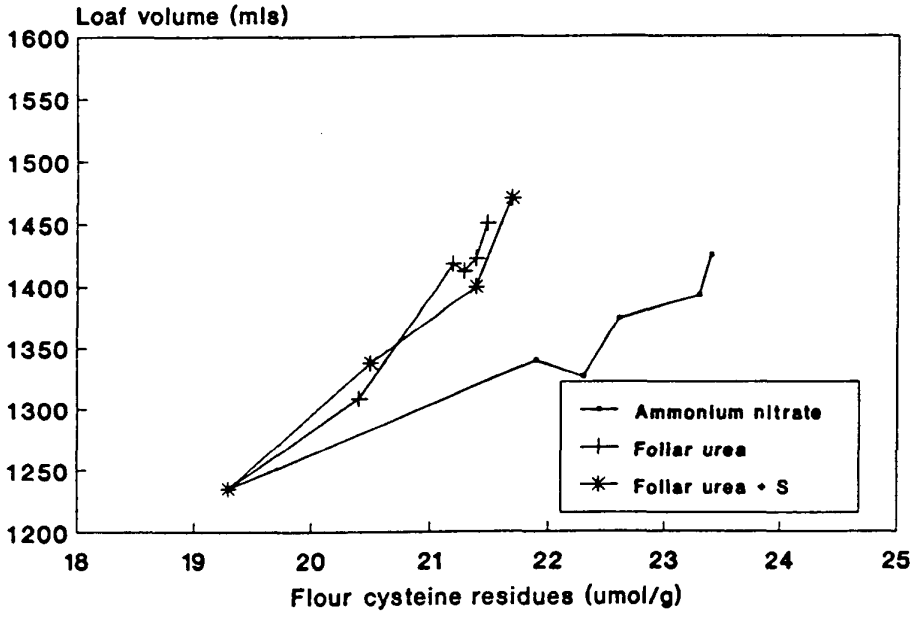


Figure 31. Effect of flour cysteine content on loaf volume (Kneesall 1988)

7.1.4 Omega-gliadins in flours

The biochemical nature, and hence baking functionality, of the additional proteins produced by extra N, was examined by estimating the relative amounts of omega-gliadins amongst the gluten proteins. These low-sulphur proteins, which are of low breadmaking quality, were conveniently assessed as the slowest moving bands in the pH 3.1 electrophoresis pattern of simple gliadin extracts from the flours. Figure 32 shows that the proportion of flour proteins in this form was increased only slightly as the rate of extra N as ammonium nitrate was increased, but it increased more than two-fold over the whole range of rates when applied as foliar urea. The enrichment of the protein with this low quality fraction and the trend towards lower amounts of omega-gliadins from foliar urea appeared to be alleviated slightly by the addition of sulphur (ns trend).

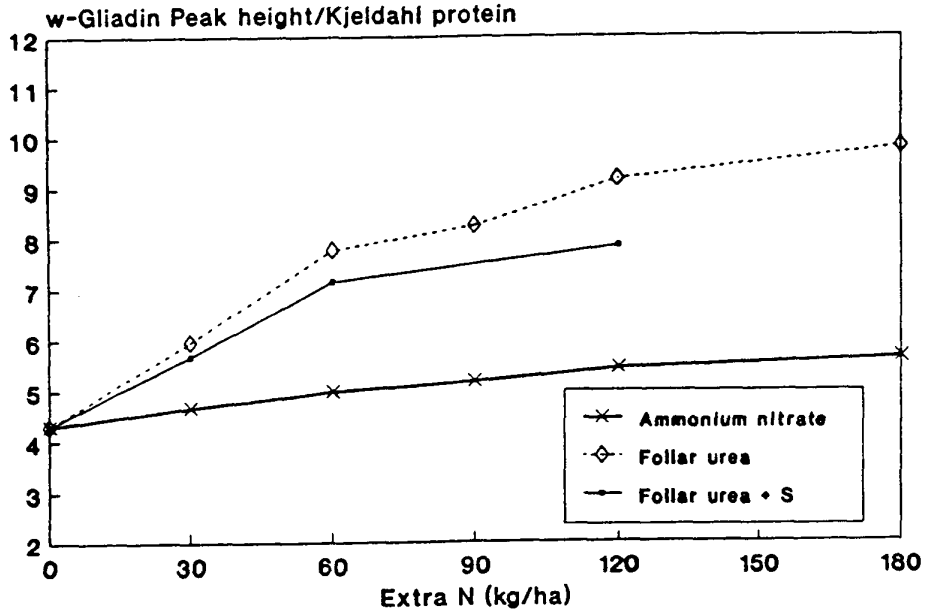


Figure 32. Effect of extra N on the ratio of omega-gliadins:flour protein content (Kneesall 1988).

7.2 Studies of protein sub-fractions from flours of Boyt (1989), Hargrave (1989), Morley (1989) and Terrington (1989)

Following the results of studies on samples from the Kneesall 1988 site, the distribution of 4 functionally different sub-fractions of the flour proteins was assessed - albumins, globulins, gliadins and glutenins. Biologically, the albumins and globulins are largely enzymes or enzyme inhibiting proteins, involved in the development of the grain, or in the protection of grain against pests and diseases. They are quite rich in cysteine residues, but do not make a significant contribution to gluten quality.

Gliadins and glutenins have evolved as the nitrogen store of the seed. Coincidentally, their structures to fulfill this role also give them the insolubility, viscosity and elasticity to form gluten, the protein mass that gives wheat flour it's unique breadmaking ability. Glutenins are rich in cysteine residues, and contribute the essential rubberiness of the dough. Gliadins, some of which are poor in cysteine residues, contribute the extensibility of the dough.

The operational protein sub-fractions chosen were an aqueous salt extract (nominally albumins and globulins), the subsequent aqueous isopropanol extract (nominally gliadins) and a final extract made with the protein solublising detergent sodium dodecyl sulphate (SDS) in the presence of the disulphide bond-breaking reagent dithiothreitol (DTT) to break up protein aggregates (nominally glutenins). A second flour sample was extracted in parallel with only the SDS/DTT solvent to give a total protein extract as a check on the sequential procedure. The extraction procedure was minimal, with only a single exposure to each solvent, so there may have been some carry-over between fractions.

The subset of samples chosen from the two sites in Trial Series A was taken from 2 replicates of 4 treatments. For each treatment, samples were taken from plots with analytical values closest to the average for the quadruplicate set. Treatments selected were the control, and 60 kg/ha of extra N applied as ammonium nitrate, foliar urea and foliar urea+S.

A similar subset of samples (2 replicates of 4 treatments) was chosen from the two sites in Trial Series B - control, foliar urea applied on day 10, 40 and 60. This range was chosen because the baking results revealed a maximum response to extra N applied on day 40.

7.2.1 Results of the protein sub-fraction and cysteine studies

For each site, the 32 protein and cysteine measurements were suitably averaged and presented as bar charts, each with four clusters of four bars. Each cluster represented a type of protein extract, and within each cluster the bars represented the different fertiliser treatments. Alongside each cluster, a line represented the LSD (5%) for that group of measurements.

Figure 33 gives an example of one of these bar charts, showing the results of the protein content of the extracted sub-fractions for the timing trial at Morley 1989.

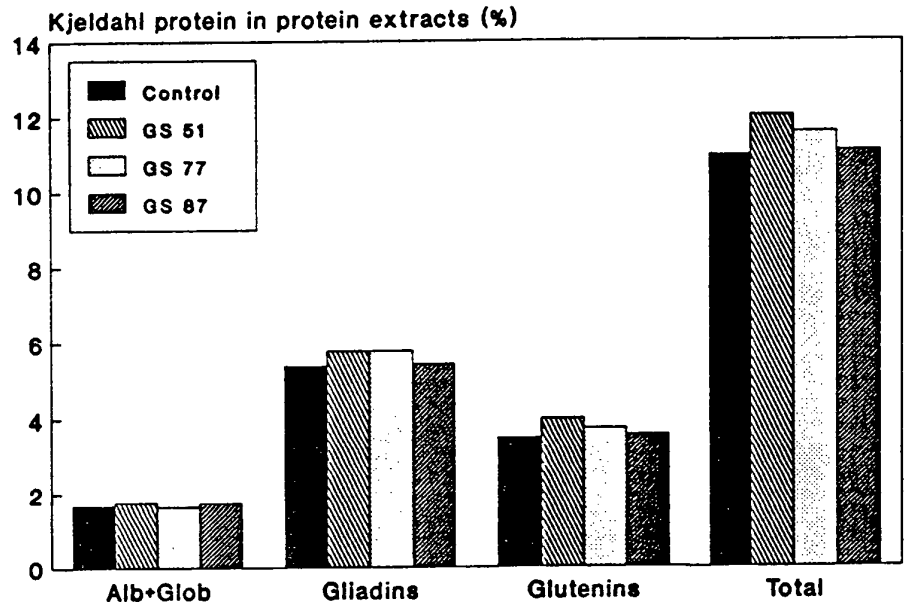


Figure 33. Distribution of protein sub-fractions in flour (Morley 1989).

Inspection of these charts showed that in no case did the protein or cysteine measurements clearly indicate differences induced by the different fertiliser regimes. They did not strongly

support, but neither did they disprove the hypothesis that use of foliar urea produced proportionately more gliadins and glutenins, and less albumins and globulins, than did extra N as ammonium nitrate. The Hargrave rate trial, a relatively poor quality site of low average loaf volume, gave the nearest to an indication of such results, but not at a significant level.

The results from the 2 timing trials showed that no systematic response to curve to correspond to the largest loaf volume response, which was found for day 40 urea application.

7.2.2 Studies of protein sub-fractions - Frampton (1990)

As this site had shown the strongest response to extra N in the 1990 harvest, all replicates of all treatments were analysed for protein and cysteine in the albumins+globulins, gliadins, glutenins and total protein fractions of the flour protein. Extraction procedures on cysteine analysis were used for the 1989 harvest, but protein analysis was done with a different procedure. This was a version of the Lowry-Folin colorimetric assay (see Section 3.2.3). Assays were run in triplicate. The meaned results were satisfactorily consistent between total extracts and the sum of sub-fractions, and with a small number of parallel estimations done by the traditional Kjeldahl procedure.

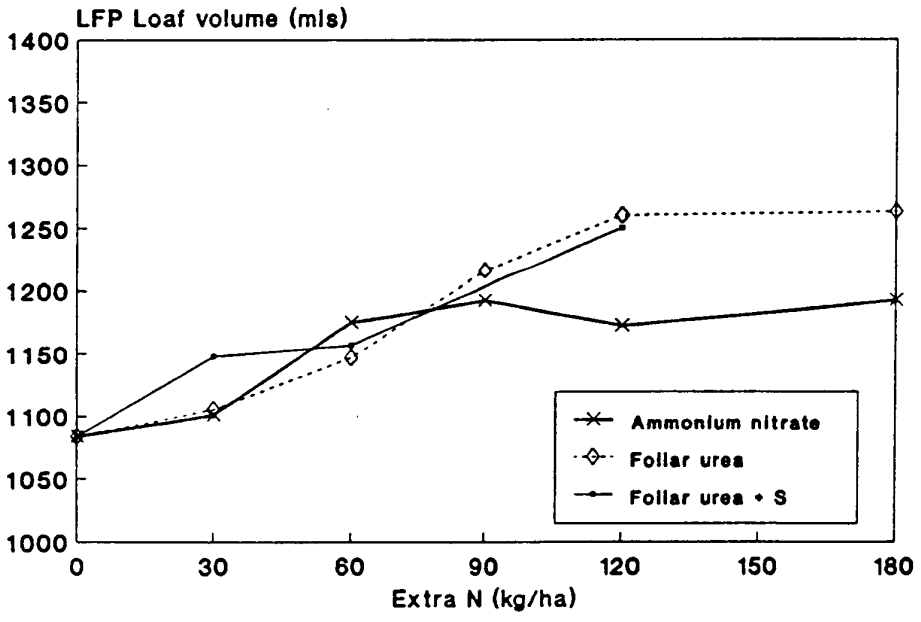


Figure 34. Effect of extra N on LFP loaf volume (Frampton 1990)

In order to put the results of the protein analysis in context, the results of Frampton 1990 for LFP loaf volume are shown in Figure 34. At the 30-90 kg/ha rates, extra N as either foliar urea or ammonium nitrate produced similar increases in loaf volume, but at 120 and 180 kg/ha rates there was only a further response from the foliar urea ($p<0.05$). This response was not affected by added sulphur.

Figures 35a-d show the protein assay results for the albumin+globulin, gliadin, glutenin and total protein sub-fractions. The lack of significant differences or even consistent trends prevents any clear conclusions about treatment differences, except for the albumin+globulin fraction at the 120 and 180 kg/ha rates of extra N. Here the higher response produced from ammonium nitrate is statistically significant. It is interesting that these are the conditions where ammonium nitrate gave a lower response in loaf volume than foliar urea (Figure 34), even though the total protein response was similar (Figure 35d).

The results of cysteine assays on the protein sub-fractions from Frampton 1990 similarly did not show significant differences between the urea and ammonium nitrate treatments, nor between the urea and urea + S, with the exception once again of the albumin+globulin subfraction at the higher rates of N input (see Figure 36). It can be seen that the fraction maintains a high level of cysteine with high levels of N from ammonium nitrate, but when the N is from urea the cysteine level decreases. The data are consistent with the hypothesis that, at high rates of extra N, ammonium nitrate tends to induce protein in the less useful form of albumins+globulins, than does foliar urea. However the data do not clearly demonstrate what sub-fraction is induced more efficiently by the foliar urea, in order to produce the greater loaf volume response.

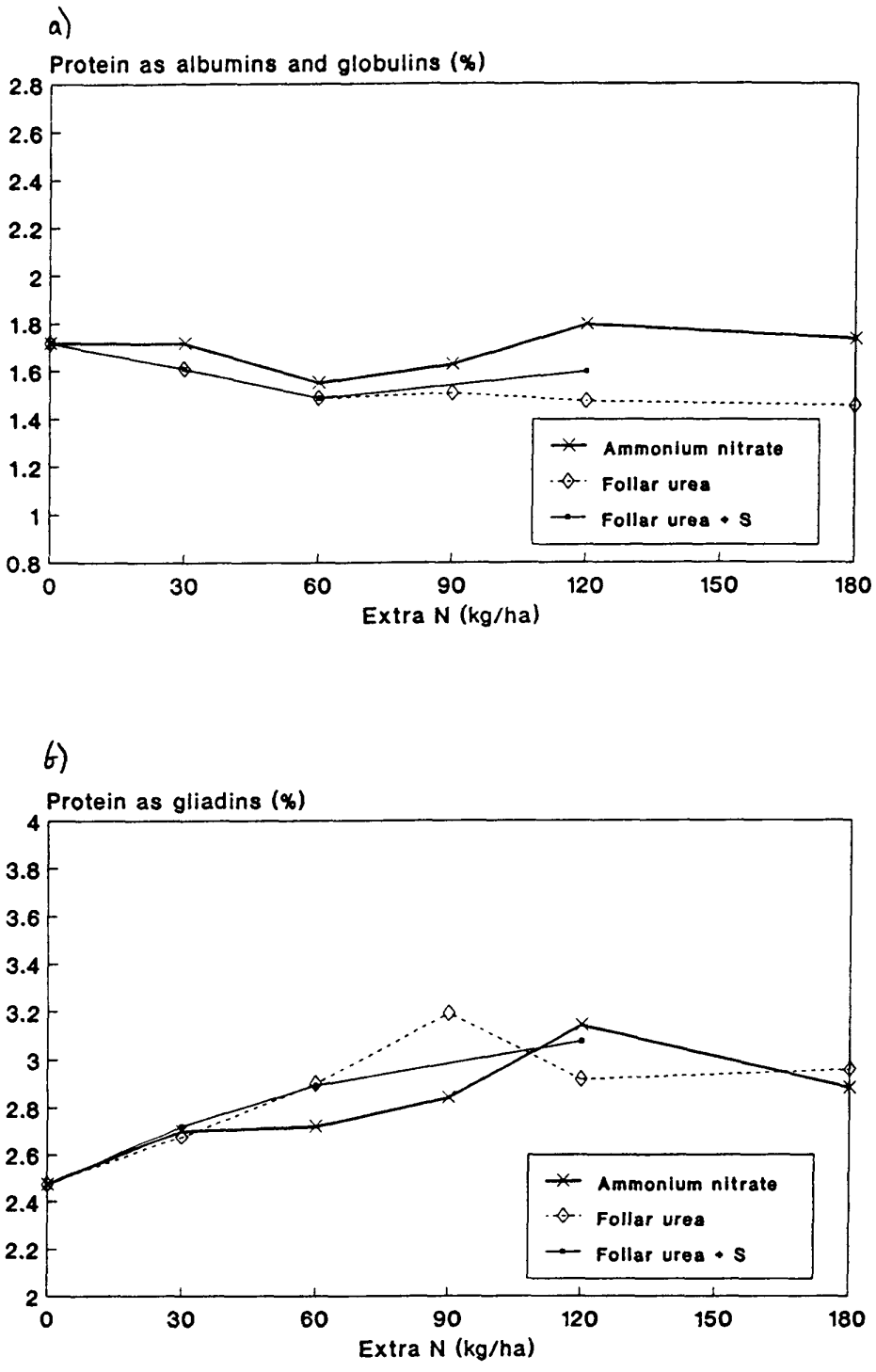
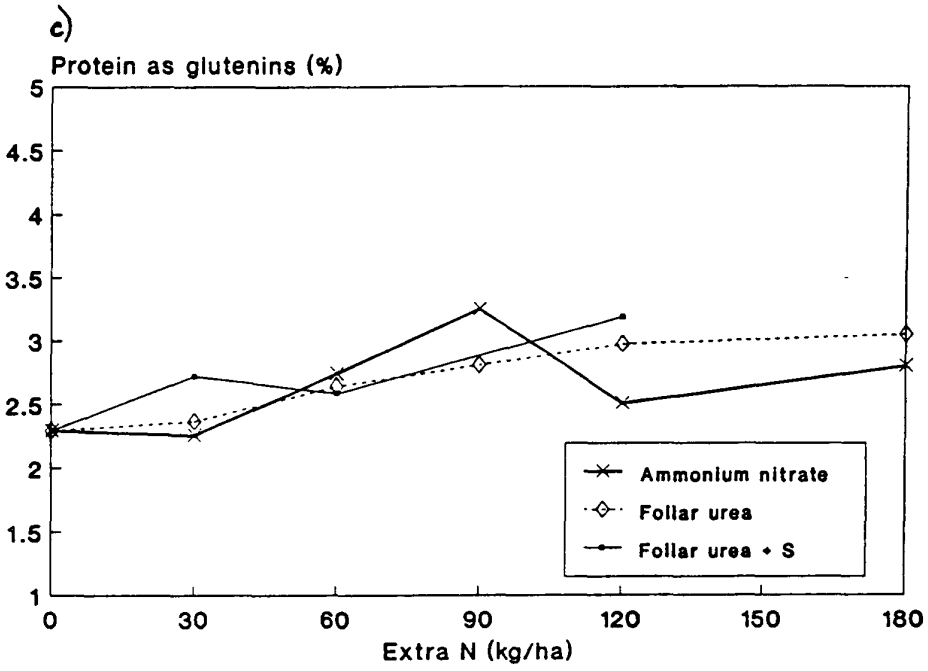


Figure 35. Effect of extra N on protein sub-fractions in flour (Frampton 1990):
(a) Albumins and globulins
(b) Gliadins
(c) Glutenins
(d) Total proteins



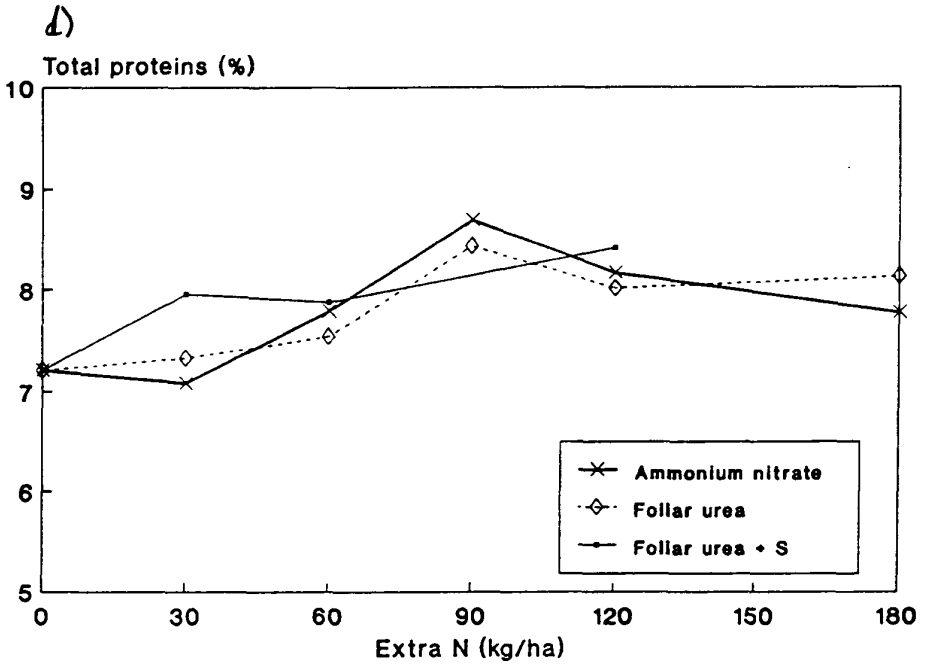


Figure 35. Effect of extra N on protein sub-fractions in flour (Frampton 1990): (Continued)

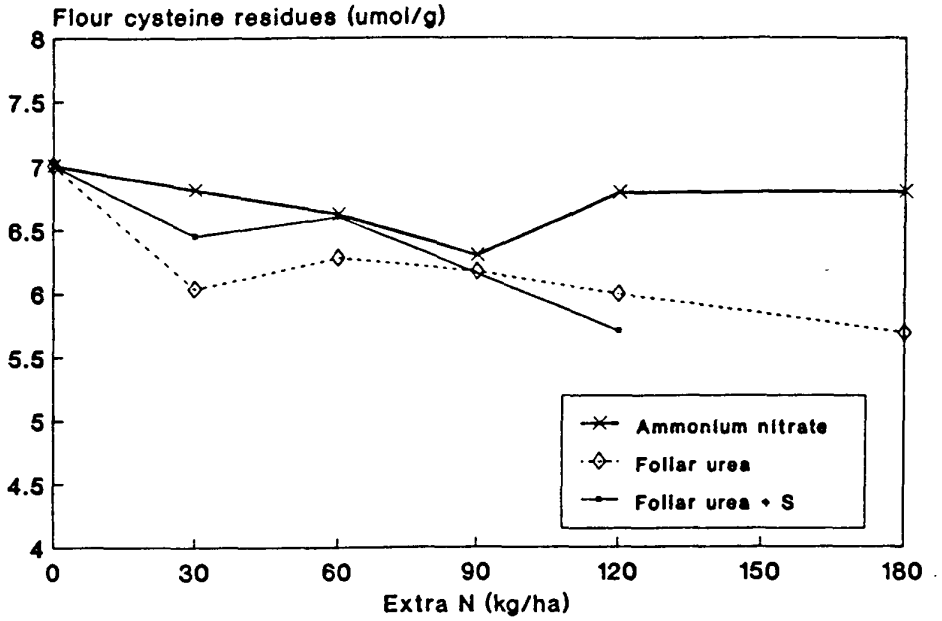


Figure 36. Effect of extra N on the cysteine content in the albumin and globulin sub-fraction of flour protein (Frampton 1990).

7.3 Studies of protein sub-fractions - Bishops Canning 1991

Since the Bishops Canning site (Trial Series A) gave the strongest response to extra N in 1991, the protein sub-fractions were examined for protein content and cysteine. LFP loaf volume data are shown in Figure 37, and the protein fractionation results in Figures 38a-d. Of the cysteine data, only the albumin+globulin fraction is presented, since significant differences were only observed in this sub-fraction (Figure 39).

Extra N as foliar urea consistently gave a higher loaf volume than from ammonium nitrate. This is a reflection of the higher total protein produced over the whole range of extra N rates. However, the data for the glutenin sub-fraction were in this case also significantly different, so it can be said that the increased loaf volume due to extra N from foliar urea is associated with extra synthesis of the high quality glutenin proteins. As with the Frampton 1990 site, the protein in the albumin+globulin sub-fraction was less from urea than from ammonium nitrate at the higher rates of extra N, but in this case the data did not achieve significance. However, the picture for the

cysteine content of this sub-fraction is statistically significant at the higher rates of extra N, and once again as for the 1990 Frampton data it shows that the cysteine levels induced by the foliar urea drop below those induced by the ammonium nitrate at the 120 and 180 kg/ha rates of extra N.

As with the preceding data, these results are consistent with, but do not prove the idea that extra N as ammonium nitrate favours synthesis of low quality albumin+globulin proteins, whereas foliar urea tends to favour synthesis of high quality glutenin proteins, particularly where the extra N is supplied at very high rates.

7.4 Gel-protein

Measurements of gel-protein were carried out on flour samples from 1990, Bishops Canning 1991 and Milton Ernest 1991. Results of the 1991 analyses are given here - essentially similar results were obtained in 1990.

Results are given in Table 19 for ammonium nitrate and foliar urea treatments only. The addition of sulphur had little effect on gel-protein properties.

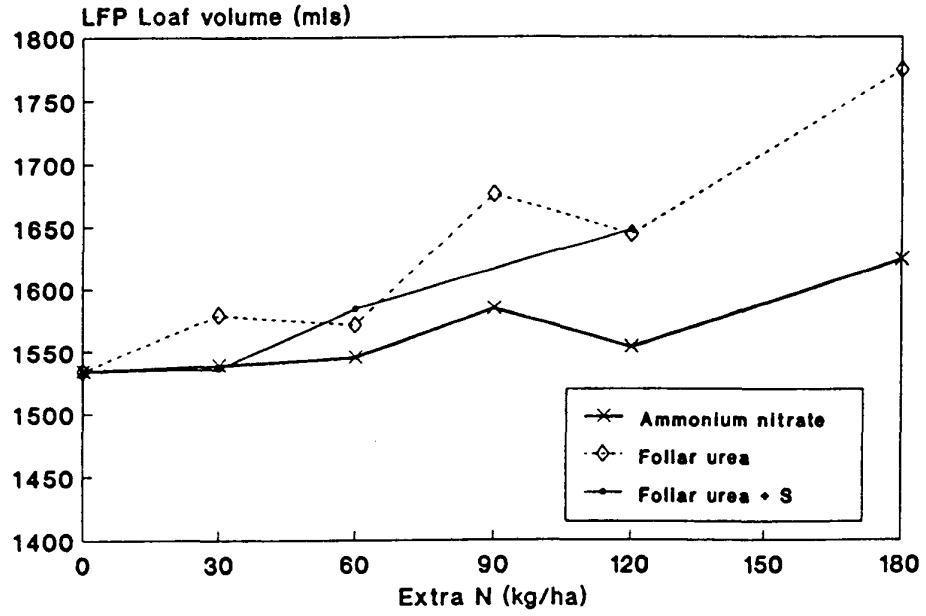


Figure 37. Effect of extra N on LFP loaf volume (Bishops Canning 1991).

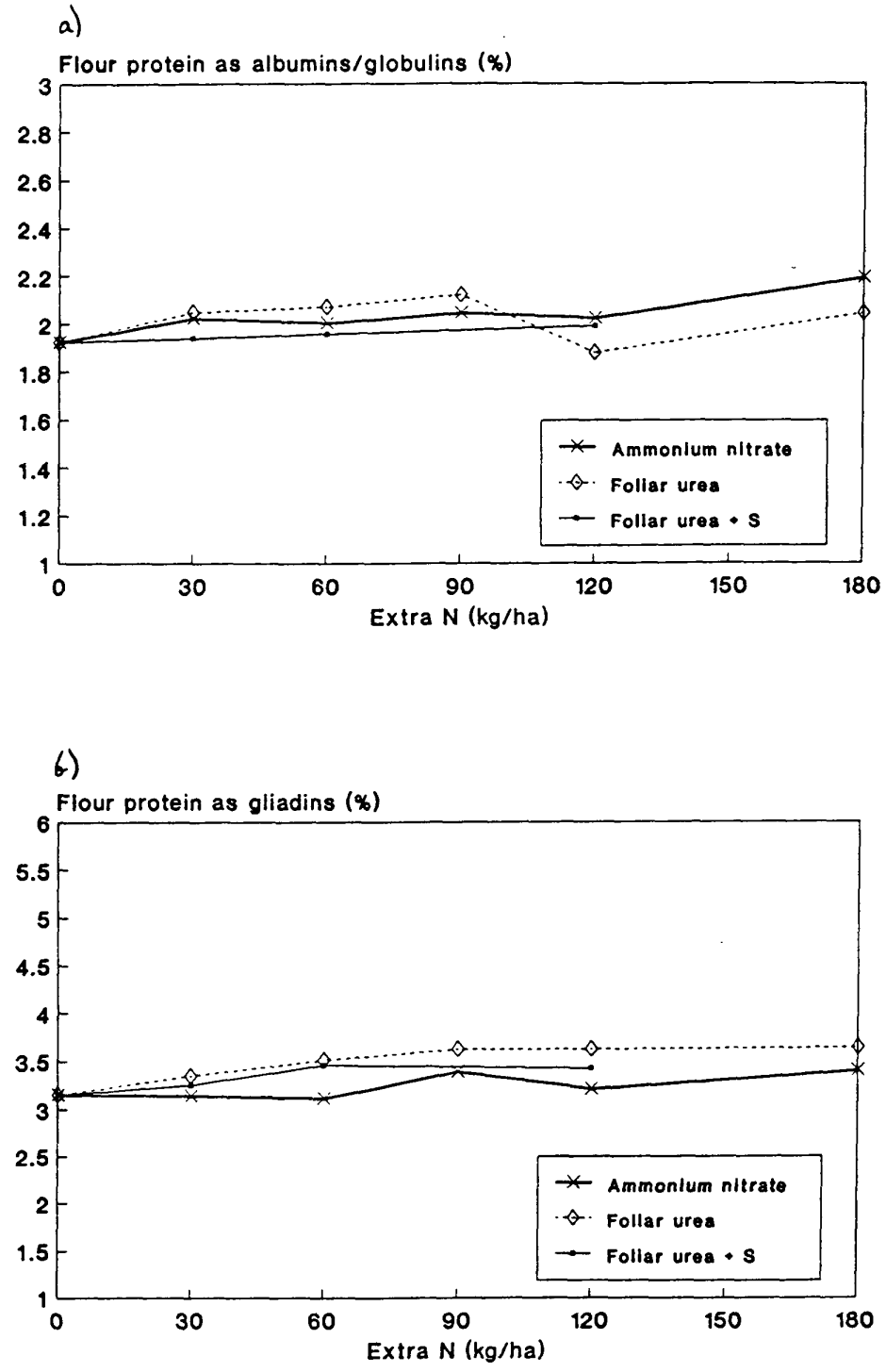
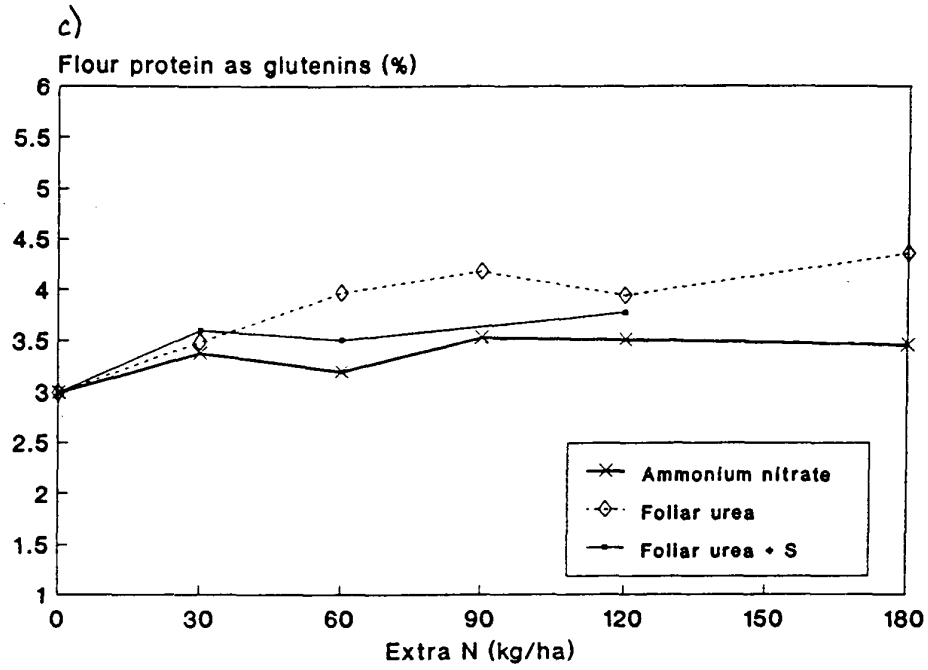


Figure 38. Effect of extra N on protein sub-fractions (Bishops Canning 1991):
(a) Albumins and globulins
(b) Gliadins
(c) Glutenins
(d) Total proteins



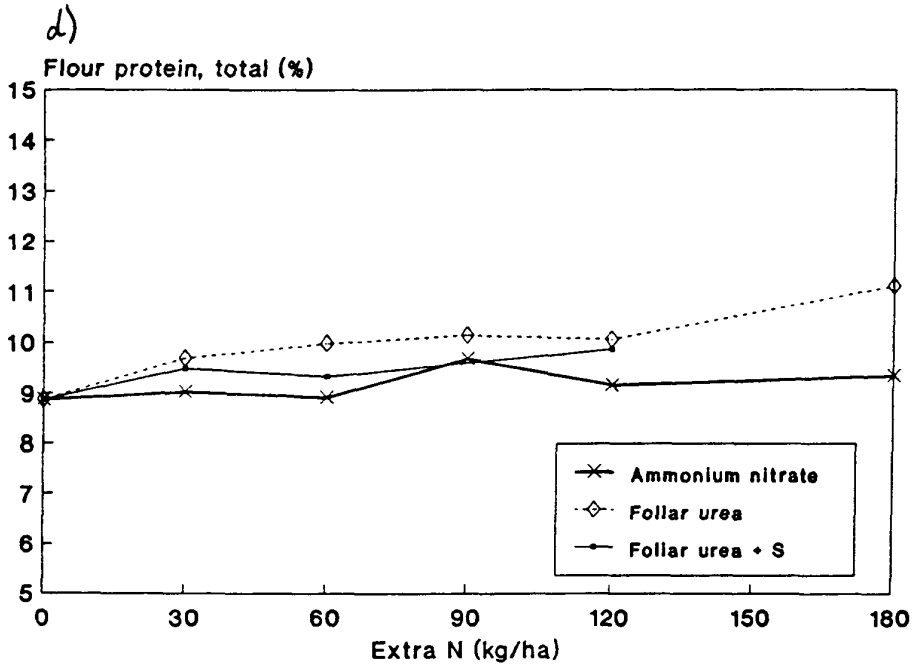


Figure 38. Effect of extra N on protein sub-fractions (Bishops Canning 1991): (Continued)

Table 19. Effect of nitrogen on the gel-protein content of Mercia flour (grams of gel-protein per 5 grams of flour)

	Rate of extra N (kg/ha N)					
	0	30	60	90	120	180
Bishops Canning						
Ammonium nitrate	11.21	10.70	11.64	11.08	11.33	11.74
Foliar urea	11.21	11.30	12.12	11.54	12.34	12.14
Milton Ernest						
Ammonium nitrate	9.83	9.42	10.66	10.47	10.77	10.65
Foliar urea	9.83	10.01	10.64	10.82	10.99	11.08

Gel-protein weights were higher at Bishops Canning than at Milton Ernest. Both forms of N increased the weight of gel-protein and there were only small differences between them. However, extra N as foliar urea tended to produce higher levels.

There were, however, marked treatment differences in the elastic modulus data (Figure 40). At both sites, elastic modulus increased approximately linearly with increasing rates of foliar urea. Extra N as ammonium nitrate had only a small effect. A similar effect was observed in samples of Mercia grown at Frampton 1990 (data not shown). At this site, G' increased from 26.4 to 43.8 when 180 kg/ha extra N was applied as foliar urea.

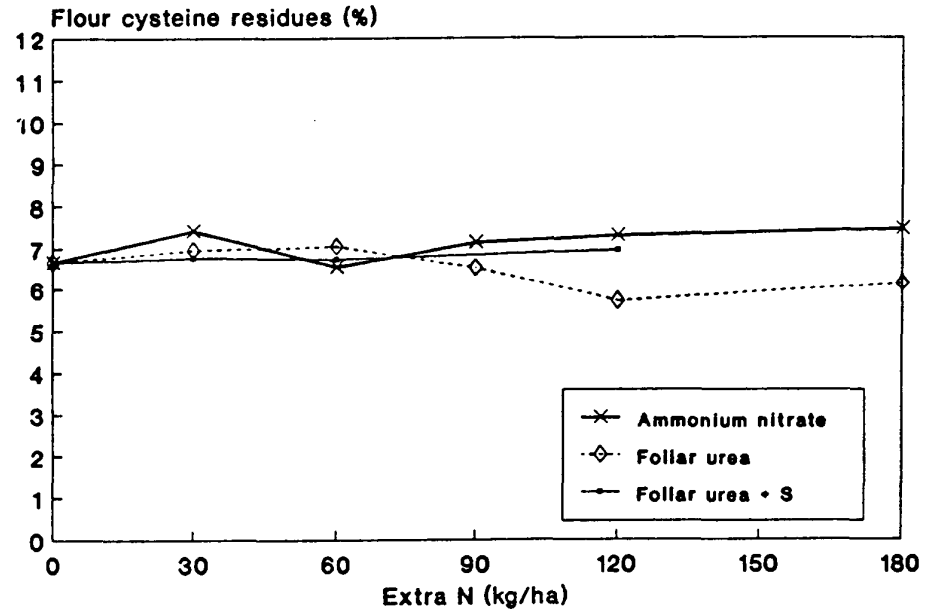


Figure 39. Effect of extra N on the cysteine content in the albumin and globulin sub-fraction of flour protein. (Bishops Canning 1991).

The technological significance of these data was studied in both LFP and CBP baking tests. The relationship between loaf volume and elastic modulus is shown in Figures 41a and b.

At Bishops Canning, there was an approximately linear relationship between LFP loaf volume and elastic modulus, which was independent of the form of extra N (r=0.94). At Milton Ernest, where elastic moduli are higher, the relationship was dependent upon the form of extra N. There was an approximately linear relationship where foliar urea was used up to a G' value of about 40 Pa.

Under the CBP baking process, extra N did not significantly increase loaf volume at either site. This may be a consequence of the ability of the CBP process to make the best use of flour protein content. Loaf volumes were higher at Bishops Canning and these higher volumes were accompanied by higher G' values. Further work covering a wider range of varieties and growing sites would be needed to establish clearly the relationship between loaf volume and elastic modulus.

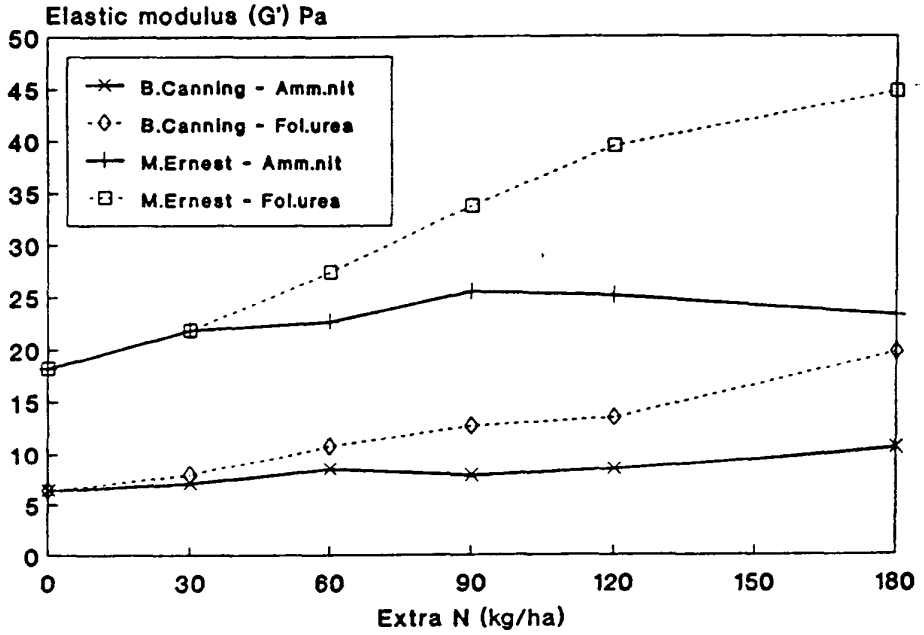


Figure 40. Effect of extra N on the elastic modulus at Bishops Canning (1991) and Milton Ernest (1989)

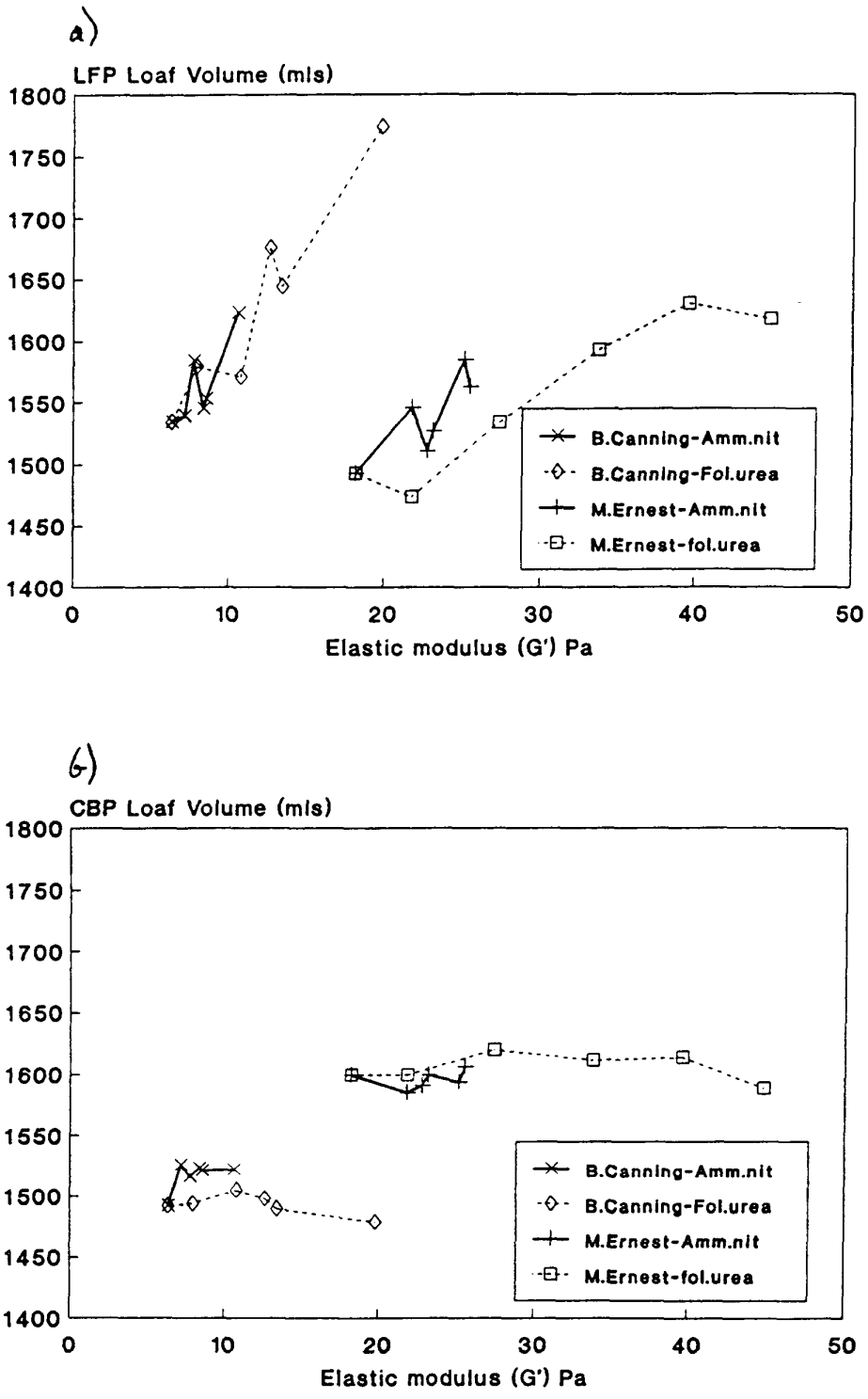


Figure 41. Effect of the elastic modulus on loaf volume at Bishops Canning (1991) and Milton Ernest (1989):
(a) LFP loaf volume
(b) CBP loaf volume

These data show that, for these sites, extra N as foliar urea has a larger effect on the elastic modulus of gel-protein than does ammonium nitrate. The evidence from test baking suggests that at Bishops Canning, there was a risk that foliar urea was producing functional protein that was too strong for normal development of bread baked by the CBP process.

These results emphasise the importance of developing measures of wheat quality that adequately predict baking performance. Further study of the elastic modulus of gel-protein is necessary to increase the understanding of this measure of flour quality which might be used to discriminate between wheats where the SDS sedimentation test lacks sensitivity.

8. DISCUSSION

8.1 Grain yield, grain quality and nitrogen recovery

Under current grain marketing structures, the financial output from a wheat crop is controlled by the level of grain yield and the price per tonne achieved by the grain. Whereas grain yield will be fixed at the point of harvest, the price per tonne can vary substantially following harvest depending on market conditions and the quality of the grain. For breadmaking wheat, premiums have varied in recent years from around £5-25 per tonne.

Because of the generally lower yield potential of current breadmaking wheat varieties, achievement of a similar gross margin compared to a feed variety will normally depend on obtaining the quality premium. This requires achievement of quality for **all** criteria - protein, SDS, Hagberg Falling Number, specific weight, moisture and impurities. Although effects on yield are important, management practices for breadmaking varieties must be geared to achieving the quality premium.

Confirming a wide range of previous work, the positive effect of extra nitrogen on grain protein content (as measured by analysis of grain N percentage) is unequivocally shown by these trials. In virtually all trials, higher rates of N use resulted in increases in grain protein, up to rates of N that are well above normal commercial practice. In Trial Series A, increases of over 3% grain protein content were commonly achieved through use of over 100 kg/ha extra N applied as foliar urea. Although such high rates are not to be recommended in practice, these effects do illustrate the potential for crop management to influence grain protein contents.

The results also show a higher capacity for grain protein where foliar urea is used. Whereas, grain protein contents of over 14% were commonly achieved from use of foliar urea, use of ammonium nitrate seemed unable to achieve proteins of more than 12.5%. The practical significance of this effect is limited however under current market requirements where the normal benchmark is at or around 11.0% protein. It may however be important for specific markets that require proteins of over 12%.

The magnitude of the protein increases varied considerably depending on the form and timing of the N application, and also the absolute protein level from which the increase was based. In Trial Series A, application of extra N as foliar urea at milky ripe stage (GS75) consistently produced a slightly larger protein increase than from application of prilled ammonium nitrate at second node stage (GS32). Across all sites, 30 kg/ha extra N increased grain protein by an average of 0.66% when applied as foliar urea (GS75), and by 0.51% when applied as ammonium nitrate (GS32). These increases varied considerably depending on the level of control protein before extra N was applied - 0.71% and 0.60% where control protein was below 10.5%; 0.67% and 0.46% where control protein was between 10.5-12.0%; 0.40 and 0.17% where control protein was over 12.0%.

It is clear therefore that it will be more difficult for farmers to produce high protein grain if this is required for a specific market, and proportionally more nitrogen will be needed by the crop to achieve this. In future considerations of grain market requirements therefore, it will be important to avoid unnecessary or over ambitious grain protein specifications.

Apart from the economic considerations of whether to grow breadmaking or feed wheats, the setting of required grain protein contents by the market has significant implications for the control of nitrate pollution of watercourses. Data on the recovery of extra N applied to increase grain protein, clearly shows that increasingly high rates of extra N are used less efficiently by the crop. The inevitable consequence is that increasingly large nitrogen residues will be left in the soil following harvest. This will increase the risk of nitrate pollution of watercourses (see Section 8.7).

In Trial Series B, foliar urea applied either too early or too late in the crop development was found to be ineffective. Although the optimum timing varied between trials, maximum effect on grain protein was usually achieved from application during GS70-79 (milk development stage). Later applications were ineffective presumably due to the onset of crop maturity, whilst earlier applications before or during anthesis were less effective probably due to the crop being in a vegetative stage of growth.

Potential adverse effects of early application of foliar urea application are also likely to be greater. Although the effects of crop scorch from foliar urea were not found to be of any great consequence, crops are likely to be more sensitive to scorch during the vegetative growth stage, and, because of the longer period to maturity, any such scorch induced is likely to have more effect than that induced at a later stage of growth.

The effects of the nitrogen treatments on grain specific weight, SDS or Hagberg Falling Number were inconsistent and generally small in all trials. They are not be regarded as important when making decisions on using nitrogen fertiliser for breadmaking wheats.

8.2 Milling Quality

Of the two components which contribute to milling performance, namely white flour extraction in a standard milling process and the colour of the milled flour, the former was not seriously affected by the amount, form or timing of extra N. This finding confirms previous work where foliar urea treatments were not found to influence flour extraction rate significantly (Hook *et al*, 1989).

A miller requires a high yield of white flour of good colour, ideally below 2.5 colour grade units. High levels of bran contamination are undesirable in white flour as this reduces the breadmaking potential. Bran has no function in breadmaking and is negatively correlated with quality within a specific grist (Cauvain, 1987). Significant increases in flour colour grade were observed in this study with increasing levels of extra N as ammonium nitrate or foliar urea, but the magnitude of such increases rarely resulted in failure to meet a typical colour specification for white bread flour.

The fixed laboratory milling system is designed to test for differences in the milling performance of wheat samples. It does not permit blending of flour streams to maintain a constant flour colour value, a facility which is available to the commercial miller. The implication of this is that high flour colour values may signal increased bran levels in some flours which would be to the detriment of their breadmaking potential. For example, at the

Bishops Canning site in 1991, grain specific weights were low (mean for control treatment 74.2 kg/hl) resulting in the production of white flour with unacceptably high colour grade values when high levels if extra N was added, eg. colour grades of nearly 5.5 were produced from the 180 kg N foliar urea treatment. The increased bran contamination of samples treated with extra N, as either ammonium nitrate or foliar urea at this site, which performs no function in breadmaking may nullify any positive effect which could result from increased protein content.

Increasing rates of extra N resulted in higher flour protein content in this work and a positive relationship is known to exist between this component and flour colour (Barnes, 1986). Starch granules, which are the major component of flour are highly light reflective and can be expected to have a reducing effect on colour values. As the protein content of a flour increases, the starch content reduces and the resulting milled flours appears darker and producers a higher colour values without an associated increase in the level of bran contamination.

Increases in flour grade resulting from increasing protein content due to fertiliser treatment have previously been observed (Hook *et al*, 1989). Since the foliar urea treatment results in higher flour protein content it is reasonable to expect more significant increases in flour colour to result from this treatment. In general, the flour produced under the laboratory system was of excellent flour colour. However, in some isolated instances the combination of high protein and slightly shrivelled grain led to to unacceptable flour colour values, even under the laboratory scale milling system. It should be noted that if flour colour values for the control treatments had been higher, the application of extra N as ammonium nitrate or foliar urea would have resulted in the miller having to sacrifice white flour extraction in order to remain within the customer's flour colour specification.

The Kent-Jones colour grader used cannot differentiate between these two factors which contribute to high colour values, namely bran and protein content. This indicates one of the problems relating to the use of this technique as an absolute measure of flour grade or quality.

Within this study, significant increases in specific weight were observed in four out of the six sites where this parameter was measures (ADAS data). Such increases were associated with increasing N rates, particularly foliar urea, but were not translated into improved flour extraction. Conversely, for some of the trial sites examined specific weights were unusually low throughout the trial, ie. Roundway in 1990 and Bishops Canning in 1991. In both trials this parameter had some effect on milling quality resulting in low flour extraction rates in Roundway trial samples and high flour colour values at Bishops Canning. Thus, gross differences in specific weight were translated into a reduction in the quantity or quality of the milled flour, but small differences had no significant effect. This confirms the poor relationship previously shown to exist between specific weight and flour extraction rate over a range of specific weights (Hook, 1984).

In the timing experiments (Trial series B) only 30-40 kg N/ha was applied as foliar urea compared with up to 180 kg N/ha in Trial series A and therefore one might expect the magnitude of any effects to be reduced. Timing of foliar urea treatment was found to have no effect on extraction rate. Some small increases in flour colour grade, average 0.4 GCF units overall were observed when extra N was applied as foliar urea, and these merely confirm the increases found in the rate trial studies and provide a more realistic view of the likely effect of commercial foliar urea applications on flour colour. Late applications of foliar urea did not produce this small increase in flour colour. Protein and breadmaking studies confirmed that applications (after GS 75) often failed to increase flour protein content ie, were not absorbed by the plant, did not result in increased protein content and consequently had no effect on flour colour. This effect confirms the observations of Barnes (1986), that increasing protein content results in an increase in flour colour values.

8.3 Flour protein content

The amount, form and timing of extra N were shown to have a significant effect on flour protein content at most sites. Within the limitations of the sites examined, foliar urea applied at GS75 was more effective in increasing flout protein content than an equivalent

level of ammonium nitrate applied at GS32. This observation was particularly valid at the highest levels of applied extra N. This confirms findings of Hook *et al*, 1989 although in a recent review (Gooding & Davies, 1992) suggests that in direct comparisons, when urea and ammonium nitrate are both applied as foliar sprays at equivalent times, ammonium nitrate may produce the greater return in terms of protein content.

During milling to produce white flour, the outer layers of the grain are discarded which results in a protein loss on milling. There was some evidence of increased protein loss when very high levels of nitrogen were applied, particularly as foliar urea. However, as a percentage of the total grain protein content, there was no evidence of a particular problem with foliar urea as this treatment consistently produced higher grain protein levels than an equivalent level of ammonium nitrate applied in the spring.

The levels of applied extra N (up to 180 kg N/ha in Trial series A) are well above commercial practice, but such high levels proved necessary to produce flour protein contents above 10.5% at some sites. In contrast, at some sites this target could be achieved without any extra N.

Protein content was measured by three different techniques in this work: summation of aminoacid data, kjeldahl and NIR. Only kjeldahl and NIR were performed on all samples selected for quality assessment. The former measures total N content (both protein and non-protein N) and converts to protein content by using a factor of 5.7 whilst the latter measures the peptide bonds in proteins but is calibrated against kjeldahl results.

The calibration samples used to establish the relationship between kjeldahl and NIR protein analyses would not contain flour samples which had received such high or such late foliar urea applications. Thus, and discrepancy between the two protein measurements, particularly if this was affected by rate or timing of treatment might indicate the presence of non-protein forms of nitrogen.

A small discrepancy was found between kjeldahl and NIR measurements in this work, but the difference was quite consistent and within the experimental errors associated with the techniques. This suggests that neither excessive levels of fertiliser N nor late foliar urea treatments (after GS79) resulted in an accumulation of significant levels of non-protein N since these would register differently in the different methodologies. Biochemical studies tended to support this view. Limited biochemical studies on samples from the Kneesall site showed that the amount of non-protein nitrogen present as low molecular weight aminoacids was insignificant.

8.4 Protein quality - fractionation and composition

Wheat is not purchased in the UK for use in a breadmaking grist on the basis of protein content alone and it is the quality of that protein, and ultimately the breadmaking performance of the flour milled from it, which is of vital importance. For nitrogen fertiliser to improve breadmaking performance, it is not sufficient for it to be absorbed by the plant, it must also be converted into proteins, particularly the large, functional glutenin type proteins crucial for breadmaking. This project has aimed to look at protein quality in several ways by SDS sedimentation volume, protein fractionation and composition studies. There have also been limited investigations of the amount and quality of gluten and gel protein.

One of the most clear cut results from the biochemical fractionation studies was the demonstration that the high levels of extra N as foliar urea selectively induced synthesis of extra protein in the form of low sulphur, low quality ω-gliadin nitrogen storage proteins (Kneesall, 1988). There was also clear data to show that ammonium nitrate induced greater levels of the sulphur aminoacid cysteine, but that it was not associated with extra baking quality, as would be expected if the cysteine were incorporated in gluten proteins.

These observations gave rise to the hypothesis that extra N supplied as spring ammonium nitrate tended to be channelled into albumin and globulin proteins, because of its earlier availability when the synthesis of these proteins started in the seed. On the other hand, late

foliar urea tended to be channelled into gluten proteins, since synthesis of them would be well underway at later stages of seed development. A corollary of this idea is that urea N would induce extra protein of higher baking quality that that induced by ammonium nitrate N, but that this benefit would be tempered if sufficient sulphur were not present to prevent excessive diversion of the extra N into the low quality ω-gliadins.

Subsequent studies, over the period 1989-1991, were aimed at obtaining data to prove or disprove this hypothesis. Sites were selected on the basis of an observed response to extra N in baking studies. Selective extraction was used to fractionate proteins into types and hence quality groups. The protein sub-fractions which roughly corresponded to categories of albumins plus globulins, gliadins and glutenins were subjected to analysis for protein and cysteine content. Cysteine is the important form of sulphur in so far as it forms cross-links within the glutenin fraction.

Either due to limitations of data or the very large differences in measured breadmaking quality which existed between the four years covered in this study, investigations carried out on flour protein sub-fractions failed to prove categorically why the extra protein from foliar urea-N produced greater LFP loaf volume than that given by ammonium nitrate (data from Frampton, 1990 and Bishops Canning, 1991 were the two absolute extremes in terms of LFP breadmaking quality in this study). However, if one extracts from the data the statistically significant differences and considers them together, then the picture which emerges is at least consistent with the original working hypothesis. There is evidence, at least for the very high levels of extra N, that ammonium nitrate led to enrichment of the cysteine-rich but poor quality albumin + globulin sub-fraction, whereas the late foliar urea led to enrichment of the high quality glutenin sub-fraction.

This latter conclusion is also clearly supported by the rheological results on the gel protein (insoluble component of the glutenin fraction). This physiochemical approach is more directly related to the functional quality of the protein in breadmaking than is the rather crude biochemical categorisation into fractions. At both Bishops Canning and Milton Ernest sites from 1991 harvest series, the elastic modulus G' of the gel protein (a measure

of protein strength) was systematically greater following urea-N than ammonium nitrate-N. This finding clearly shows enhanced production of the high quality large glutenin aggregates under the influence of urea.

The tentative conclusions expressed above are in general agreement with earlier work in the UK by Timms *et al*, 1991. In addition, a large body of literature exists on the use of foliar urea under a wide variety of conditions. An extensive review of this literature has been published recently (Gooding and Davies, 1992). No attempt has been made in this discussion to explore the wider context; the published review should be consulted instead.

8.5 Breadmaking quality

A key objective of the project was to establish if additional protein produced by extra N is functional, ie, is translated into an improvement in breadmaking performance, Some doubt has been cast on the relationship between these two parameters in previous work (Hook *et al, 1989)* and (Sylvester-Bradley, 1990).

For the LFP breadmaking procedure, used in most of this work, the amount, form and timing of fertiliser N treatment was found to have a significant effect on loaf volume and score. Extra N, applied as foliar urea, was more effective in terms of increasing both flour protein content and breadmaking quality than an equivalent amount of ammonium nitrate. On the assumption that a straight line relationship exists between flour protein content and loaf volume, the slopes of fitted lines for foliar urea and ammonium nitrate were not significantly different. The foliar urea merely appears to be more effective because it continues to produce additional protein above that produced by an equivalent ammonium nitrate treatment.

Therefore, within a site there appears to be a reasonable relationship between flour protein content and LFP loaf volume, but seasonal variation in breadmaking performance often outweighs the differences achieved by the application of substantial quantities of fertiliser N. For example, the highest and lowest flour protein content samples did not necessarily

produce the highest and lowest quality bread. This illustrates the importance of conducting field trials over several harvest years in order to obtain a balanced picture of the effects of agronomic practises on end-use quality and of the importance of end-use quality. Simply measuring quality against standard intake tests like protein content, SDS sedimentation volume and Falling number only indicates whether a sample will be accepted at mill intake, not how it will perform in the bakery.

In addition, there remains the question of differences in breadmaking performance between the LFP and CBP baking methods. These two methods are very different in their recipe and processing details. The LFP method uses long fermentation and slow acting improvers to condition the dough ready for baking. The CBP method uses a fixed amount of intensive mixing and relies on the reduction of ascorbic acid in the mixing bowl to form the oxidisation improver dehydroascorbic acid, which then acts relatively quickly to help produce a fully developed dough.

Unfortunately, direct comparison was only possible for two sites in 1991 which were both characterised as producing high protein contents, but lacking in some other quality aspects. For Bishops Canning, wheats were slightly shrivelled and produced high flour colour values on milling. The consequent increase in bran levels in the white flour is likely to have negated any potential positive effect of increasing protein content. This effect is likely to be more apparent in the CBP type process, where the bubble structure produced during intensive mixing is critical for end quality. At Milton Ernest, nitrogen application resulted in high protein samples of excessive protein strength which is likely to affect their performance in a standard CBP baking process.

The variety Mercia receives a breadmaking quality classification of 6 on the NIAB Recommended List of Cereal varieties (Anon, 1993). This means that the variety is capable of producing CBP bread of satisfactory, but not the highest quality. Loaf volumes (400 g bread) of above 1500 ml are considered to be good and above 1600 ml to be exceptional for this variety. Therefore, for the 1991 sites, Mercia may have reached its

plateau as far as breadmaking quality is concerned and further improvement may have been unlikely.

During the course of this project FMBRA's knowledge of the performance and quality characteristics of the varieties under examination has increased considerably due to other HGCA funded project work. (Pritchard *et al*, 1992) and (Salmon *et al*, 1994). In particular, the revised testing protocol (Osborne *et al*, 1991) used to assess new UK wheat varieties provides the opportunity for a more thorough investigation of protein quality. It has become apparent that the variety Mercia, in particular, does not respond well to increases in protein content as some other varieties, eg. Hereward, in terms of CBP breadmaking quality. The slope of the line relating protein content and breadmaking quality is not as steep for Mercia as it appears to be for some other varieties. This confirms observations in this project where fertiliser treatments applied to Mercia grown in high protein situations did not always produce significant improvements in breadmaking quality, particularly using the CBP method.

In addition, gel protein studies carried out in the later stages of this project only, have shown that foliar urea has a more significant effect on the elastic modulus of the gel protein fraction. This elastic modulus (G') provides a measure of the strength and quality of the gel protein. Experiences with UK (Salmon *et al*, 1994) and continental wheats (Pritchard *et al*, 1992) have shown that varieties which have G' values below 10 or above 40 Pa do not produce optimum performance in a CBP test baking systems. In particular, wheats with very strong gel protein (ie. high G') will not be fully developed in a standard CBP breadmaking system where a work input of 11 watt hours/kg is used. Within this project foliar urea treatments produced very high G' values at two of the three sites studied: high protein Milton Ernest and low protein Frampton site. Such high G' values could have limited the breadmaking potential of wheats receiving substantial foliar urea treatments at these sites.

It is obvious from this study that the underlying quality of wheat differs from one season to another. Results of baking tests performed on Trial Series A, where only the variety

Mercia was tested, shows that significant seasonal variation in quality exists for this variety as well as some site-to-site variation. The magnitude of season-to-season variation in performance (see Table 14), can be seen to exceed the effect of fertiliser treatment.

In particular, Mercia grown at Frampton and Roundway in 1990 did not achieve the performance expected of this variety when flour protein was increased to above 10.5% by fertiliser treatment. This phenomena has been investigated as part of a separate HGCA funded project in which the effects of seasonal variation in the quality of breadmaking wheat has been studied in some detail over the harvest years 1991-1993, in an attempt to elucidate the underlying biochemical cause of such differences. Considerable variation in CBP breadmaking quality, though not as extreme as that found in this project where the LFP baking test was used, was observed between the three harvests 1991, 1992 and 1993. In each year, samples were selected on the basis of having satisfactory content and Falling Number for breadmaking and, therefore, there was not the extremes of protein content observed in this study.

However, seasonal differences in protein content did exist and, more importantly, biochemical studies have indicated differences in the quality of the protein laid down. In a season when protein content is lower than average there appears to be a compensation in terms of the strength of that protein. In particular, the elastic modulus of G' appears to increase when protein content decreases. Other differences in the composition of the protein laid down are still being investigated. However, it is possible that the combination of the effect of the season on quality, ie. generally low protein content in control treatments in 1990 (which evidence suggests one might expect to be of increased strength, ie. have a high G') and increasing fertiliser treatments (which observations from 1991 suggest result in a further increase in G') may result in wheat which fails to achieve optimum performance in a CBP baking test.

8.6 Nitrogen strategies for breadmaking wheats

Farm selection of the best strategy for nitrogen application to breadmaking wheats is a difficult issue. The trials have clearly shown that extra N above normal rates will increase grain protein however it is applied. However use of extra N will only be economic if a quality premium is obtained, and currently this cannot be guaranteed, nor it's financial value known.

Having decided to grow a breadmaking wheat, farmers should firstly apply optimum rates of spring nitrogen fertiliser for yield, avoiding excess amounts applied at the early spring timing. The next decision is whether any extra N is justified to improve grain protein. At present this can only be based on a forecast each season of the scale of likely quality premiums, and on the farm history of success in achieving quality specifications. However, because of the current large differences in yield potential between breadmaking and feed varieties, farmers are unlikely to be growing breadmaking wheats unless these specifications are realistically achievable.

Previous work (Sylvester-Bradley and George, 1987) has concluded that extra N is commonly justified, but provided other quality parameters can be reliably achieved. If achieved, a quality premium of £15 per tonne represents an extra return of £120/ha for a crop yielding 8 t/ha. With higher premiums, the returns can be higher. This extra return is essential for breadmaking wheats to compete against feed wheats.

Since the cost of 30 kg/ha extra N is about £15/ha including spreading, the proposition for using extra N looks attractive at these premium levels, even though on average, use of extra N will only achieve an actual increase in the premium paid in about 1 out of 4 crops. For the other 3 crops, the increase in protein will not alter the premium level either because grain protein continues to remain below the minimum threshold, or because it is already above that required for the maximum premium band. The cost of the extra N used on these crops must, however, be taken account of.

Based on this 1 in 4 success rate, the real cost of the extra N, therefore, is £60 for every hectare of crop where this rate of extra N is successful in achieving an increased premium. For a yield level of 8 t/ha, a premium of £7.50/t will be needed to cover this cost.

When applying extra N, 2 alternative approaches have been compared in this project:

i) Ammonium nitrate prills at or around GS32 (second node stage)

ii) Foliar urea spray between GS70-79 (milk development)

There are pros and cons for each of these approaches. Foliar urea will consistently give a slightly higher protein content but the difference from 30 kg/ha N is only about 0.2% on average. For an individual crop it is not possible to predict the importance of this difference. Prior to the work reported here, there has also been uncertainty concerning the breadmaking value of the protein produced by late foliar urea sprays. Evidence from this Project shows that late foliar urea does produce useful protein for breadmaking.

In addition to extra protein, extra N as ammonium nitrate may also give a small yield benefit which is unlikely from foliar urea unless spring N rates are drastically underestimated. This yield benefit has been shown in the current trials but would also be expected from N applied during stem extension because of the way that nitrogen recommendations are defined - ie. as 'optimum economic rates'. Thus, if 30 kg/ha extra N is used above the recommended rate for yield, a small yield increase of about 0.07t/ha (Chambers, pers. comm) would be expected, though not enough to cover the cost of the extra N used. Based on grain at £100/tonne, the value of this would be about £7/ha on all fields receiving extra N, or £28 for every hectare of crop that crossed an 11% threshold premium. Lodging risk, however, may be higher from this application approach.

Whilst there continue to be uncertainties over premium levels and the success rate of using extra N, there are attractions to applying extra N as ammonium nitrate prills at GS32. There is little difference in the protein response, and the likelihood of extra yield will go a long way to recovering the cost of the extra N. It is concluded that this approach will be best where premium levels are anticipated to be low/moderate, an 11% protein target is set and lodging risk is low.

However, as grain prices fall leading to a lower value of any yield increase, and if premium levels remain firm and/or more consistent, extra N as foliar urea will become more attractive. Foliar urea will also be the preferable option where there is a significant risk of lodging from an earlier application of extra N.

Of particular importance for the industry is the need to develop a technique that will allow a pre-harvest prediction of grain protein content. This would hopefully allow the use of a prescribed rate of extra N to achieve a specified grain protein content. It may then be possible to recommend rates above the current conventional 30-40 kg/ha N. If such a technique can be developed, it will allow a closer targetting of the grain protein content of individual crops to market demand. This will encourage much more efficient use of extra N than at present, improved crop management efficiency, improved control of nitrate pollution and a likely emphasis on the use of late foliar urea sprays with rates of application controlled by crop assessment and market demand. Some promising work on pre-harvest prediction has been carried out but more research is needed (Sylvester-Bradley, 1987).

8.7 Implications for nitrate leaching and pollution

The effects of high N use on the potential for nitrate leaching and pollution of watercourses is of crucial importance. The use of extra N for breadmaking wheats is of particular concern because of the susceptibility of this application to losses by leaching. It is now well established that any N used above the optimum rate for yield is at high risk of being lost by leaching after harvest (MAFF, 1993). The use of extra N for purposes of increasing grain protein content will usually fall into this category.

Data on the recovery of the extra N applied in these trials emphasises that only a small proportion of the application is taken up by the crop. In spite of the large increases in grain protein content through increasing rates of extra N, Table 6 clearly shows that the actual amounts of extra N taken up by the crop are only small, even from low application rates. The balance of the application rate will be mostly left in the soil and crop debris and will be at high risk of being leached into watercourses.

Where extra N is used for breadmaking wheats, the quantity of unused N left in the soil and crop debris from a normal farm application rate of 30 kg/ha extra N, will amount to about 20 kg/ha N (66% of the application) with little difference if it is applied as prilled ammonium nitrate (GS32) or foliar urea (GS75). This is a significant extra residue when considered in the context that in a

normal winter of 250 mm excess winter rainfall typical of lowland arable areas of Central England, leaching of 28 kg/ha N will theoretically result in a level of nitrate in drainage water of 50 mg/litre of nitrate, the EC limit for drinking water.

Such nitrogen residues emphasise the crucial importance of careful targetting of extra N so that application is only made to crops where a positive benefit is expected in meeting market demand. Selection of appropriate application rates and timings is equally important to maximise the efficiency of N use and to minimise residues.

8.8 Effect of sulphur application

In both Trial series, sulphur was applied with the intention of increasing grain sulphur content and thereby potentially influencing grain yield and quality. However, most of the sites were on typical wheat growing soil types or in areas where sulphur deficiency would not be expected. Examination of the sulphur response of breadmaking wheats was not a priority aspect of the project though has increased in importance during the life of the project.

In 1988 and 1989, a proprietary micronised elemental form of sulphur was used as the source of sulphur applied as a foliar spray at GS 75. In 1990 and 1991, additional water soluble sulphur (50 kg/ha S applied in early spring) was also used in an attempt to ensure an adequate supply of plant available sulphur. This followed the results of parallel work by McGrath (19) indicating the poor effectiveness of elemental sulphur applied as a foliar spray.

Based on grain sulphur analysis, few sites would be classed as sulphur deficient. There was only one site (site 30, Detling 1988) that would be classed as deficient based on the criteria of below 0.12% total S in grain dry matter and an N:S ratio over 17, but 12 sites would be classed deficient on a criteria of an N:S ratio alone of over 17 (Byers et al, 1987).

Responses of grain yield and quality parameters are summarised in Appendices 8 and 14. Although a range of statistically significant responses were found, most of these were unconvincing and erratic. At 18 sites there was a total of 24 statistically significant responses of

grain yield and/or one or more grain quality parameters to sulphur application. However, only 11 of these responses represented an improvement in the parameter.

At 3 sites, there were significant yield responses to sulphur but at 2 of these, grain sulphur levels were well above deficiency levels. Grain protein content was increased by sulphur at only one site (associated with a decrease in yield but increase in SDS), but was decreased at 3 sites. Grain SDS volume was increased at 3 sites. In all seasons, the effect of sulphur on grain sulphur concentrations was non-existent or very small irrespective of the source of sulphur (Appendix 15).

Sulphur had no consistent effect on milling quality. Applications of sulphur can act as a mild fungicide, which might be expected to help maintain grain filling and reduce the possibility of foliar disease, which could have implications for the components of milling performance, ie. flour extraction and flour colour. Neither parameter was affected significantly by sulphur treatment.

The limited and varied responses to sulphur in these trials may be due to unresponsive sites or ineffective methods of sulphur fertilisation. The lack of response reported here should not be taken to indicate a lack of sulphur deficiency in other situations.

8.8.1 Sulphur - protein content and quality effects

Sulphur treatment had no significant effect on flour protein content. There was a trend (ns) for slightly higher protein content when sulphur was applied with urea-N.

Biochemical studies provided little extra information on the related question of the S content of the protein produced by extra N as foliar urea, particularly as it affects the relative synthesis of low quality gliadins and high quality glutenins. The initial work on the 1988 Kneesall site gave hints that S supplied with urea was beneficial, but the effects did not reach significance. The later work did not give such hints, and the physiochemical studies on the 1991 harvest were no help in this regard, since no effects of the extra S were seen there. Also, throughout the study no clear effects of the extra S were seen in the breadmaking results.

9. CONCLUSIONS

1. Substantial increases in grain protein were found from extra N (above that applied for yield) applied as ammonium nitrate at GS32 (2nd node stage), or as foliar urea applied at GS75 (milky ripe stage).

2. Protein increases were greater from foliar urea and at most sites continued up to the highest rate of extra N tested (180 kg/ha N). Protein increases of over 3% protein were found at some sites. Protein increases were larger where the base level of protein was low.

3. Effects of treatment on grain yield were low, though at responsive sites, yield increases were greater from extra N as ammonium nitrate.

4. The recovery of extra N in grain was low (33% of a 30 kg/ha N application) and reduced as the rate of N increased. The recovery of extra N applied as foliar urea was commonly higher than when applied as ammonium nitrate.

5. There were small and inconsistent effects of extra N on specific weight, grain SDS and Hagberg Falling Number.

6. The effects of sulphur treatment were infrequent and erratic. However, the trial sites were not selected to be prone to sulphur deficiency and more work is needed on sulphur responses of breadmaking wheats.

7. Application of extra N resulted in an increase in flour colour grade, particularly when high levels of foliar urea were applied. Increases were rarely of sufficient magnitude to cause milling problems and could, at least partly, be attributed to observed increases in protein content.

8. Extra N applied as foliar urea at GS75 was consistently more effective in increasing protein content than an equivalent rate of extra N as ammonium nitrate at GS32.

9. Responses in LFP breadmaking performance were similar from extra N applied as ammonium nitrate or foliar urea. Responses in loaf volume were greater where foliar urea was used since this treatment produced a greater response in protein content.

10. Due to limited data it was not possible to confirm whether effects obtained for the LFP breadmaking process could be translated into the CBP.

11. All the evidence suggests that foliar urea, applied at or before GS75, is fully absorbed by the plant and converted into proteins which contribute to breadmaking performance.

12. Timing of foliar urea spray applications is important. Applications after GS75 may not be absorbed by the plant, may be too late to increase protein content and consequently have no effect on breadmaking quality.

13. Responses in breadmaking performance to sulphur applications were small and insignificant in this work. This may be attributed to poor uptake of the sulphur sources used in this work or to the fact that the sites examined were not sulphur deficient.

14. Biochemical studies suggested that foliar urea spray applications may result in enhanced production of the large glutenin aggregates which play a vital role in breadmaking. However, in some seasons the combined effect of very high fertiliser levels and inherently strong gluten protein may result in wheat which does not perform to its full potential in a standard breadmaking process.

10. RECOMMENDATIONS FOR FURTHER RESEARCH

1. It is essential that progress is made towards identifying crops which would benefit most from additional N fertiliser treatments in order to avoid unnecessary N applications and to ensure that the farmer achieves the maximum return. A means of assessing the growing crop for grain protein potential at harvest is urgently required.

2. Further research is required to establish the most effective form and optimum timing for sulphur fertilisation to improve grain quality and baking performance. Work by McGrath et al has indicated that sulphur deficiency has increased in the UK over the last 10 years and this has prompted support from HGCA for a project entitled "Evaluation of the yield and breadmaking quality responses of winter wheat to sulphur fertiliser application" which aims to identify efficient fertiliser practices which increased the sulphur content of the grain.

3. Basic wheat intake tests are not sufficient to predict final breadmaking quality. Ideally all projects designed to produce "quality" for particular end-use (eg. bread, biscuits, etc) should be examined in terms of their performance in the particular product.

4. In the past the SDS sedimentation test has proved satisfactory in the UK for breeding and wheat quality assessment. Work carried out within this project and the Recommended List testing scheme confirms that the test is just not sensitive enough to discriminate between modern breadmaking wheat varieties. Limited gel protein studies, carried out within this project, have indicated the possible value of this as predictor of performance in a CBP baking process. A combination of gel protein weight (which provides similar information to the SDS test) and the elastic modulus or G' (which provides a measure of gel protein strength) yields more valuable information on the breadmaking potential of a wheat sample. At present the test requires sophisticated equipment such as a rheometer and ultracentrifuge. It is vital that work is carried out to modify the procedure to one that is more practically applicable to all sections of the grain trade.

5. This project has helped to restore miller's and farmer's confidence that appropriate levels of foliar urea applied at the right time can result in benefits to both parties. However, our

knowledge of the expression of breadmaking quality is limited and the effect of N fertilisation on critical events in the developing wheat endosperm in unknown. The influence of nitrogen fertilisers on the expression of functional proteins in wheat is currently being studied under a new HGCA grant.

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APPENDICES

APPENDIX 1. Trial Series A. Grain yield (t/ha at 85% DM)

Summary of responses (significant differences $p<0.05$).

Site	Control yield (t/ha)	Control v all N trts	Effect of N rate	AN>U (all rates) (t/ha)	AN * U interaction (all rates)	Sulphur (t/ha)
1.	7.55	ns	*	+0.28***	*	+0.13*
2.	7.20	+0.43*	**	ns	*	ns
3.	6.89	ns	ns	+0.34***	ns	ns
4.	No data					
5.	4.84	+1.72**	ns	+0.97**	ns	ns
6.	7.20	+0.18*	ns	ns	**	ns
7.	5.54	ns	ns	ns	ns	ns
8.	8.99	+0.25***	*	+0.09***	*	−0.22***
9.	8.36	ns	ns	+0.24*	ns	ns
10.	6.60	ns	ns	ns	ns	ns
11.	4.57	ns	ns	−0.24***	ns	−0.15ns
12.	9.27	ns	ns	ns	ns	ns
13.	8.04	ns	ns	+0.62***	ns	+0.36*
14.	6.82	+0.17*	*	+0.16***	ns	ns
15.	5.15	ns	ns	ns	ns	ns
16.	7.81	ns	ns	ns	ns	ns
17.	8.92	+0.27**	***	ns	ns	ns
18.	7.82	+0.36*	ns	ns	ns	ns
19.	7.25	ns	ns	ns	ns	ns
20.	6.15	ns	ns	ns	ns	+0.20*
21.	7.42	ns	***	+0.14*	*	−0.12ns
22.	7.18	ns	ns	ns	ns	ns
23.	No data					
24.	7.68	ns	***	+0.14***	***	ns

Responses to sulphur:

Site 11 significant at p=0.077
Site 21 significant at p=0.059

APPENDIX 2. Trial Series A. Grain protein (% at 86% DM).

Summary of responses (significant differences $p<0.05$).

Site	Control protein (%)	Control v all N trts	Effect of N rate	U>AN (all rates)	AN * U interaction (all rates)	Sulphur (%)
1.	10.39	***	***	***	***	ns
2.	8.65	***	***	***	*	ns
3.	9.20	***	***	***	ns	ns
4.	No data					
5.	8.60	***	***	*** (AN>U)	ns	ns
6.	7.96	***	***	***	***	ns
7.	11.65	***	***	***	ns	−0.55*
8.	9.16	***	***	***	ns	+0.17*
9.	10.53	***	***	***	ns	ns
10.	9.87	***	***	**	*	ns
11.	13.13	***	***	ns	ns	ns
12.	12.35	***	***	***	*	ns
13.	11.02	***	***	***	*	−0.22*
14.	10.50	***	***	***	*	ns
15.	11.64	**	ns	**	ns	ns
16.	8.89	***	***	ns	ns	+0.38ns
17.	10.03	***	***	***	**	ns
18.	9.56	***	***	***	ns	ns
19	12.99	***	**	***	*	ns
20.	11.88	***	***	***	***	−0.33*
21.	12.25	***	***	***	***	ns
22.	12.97	***	ns	***	***	ns
23.	No data					
24.	12.32	***	***	***	***	ns

Responses to sulphur:

Site 16 significant at $p=0.083$

APPENDIX 3. Trial Series A. Apparant recovery of N in grain (%).

Summary of responses (significant differences p<0.05)

Site	N recovery % (mean of all rates AN	U	Effect of N rate	U>AN (all rates)	AN * U interaction	Sulphur
				%		%
1.	9	10	–	ns	ns	+5ns
2.	30	34	–	ns	ns	ns
3.	33	33	–	ns	ns	ns
4.	No data					
5.	76	37	–	-40***	ns	ns
6.	33	50	–	+17***	*	ns
7.	11	15	ns	ns	ns	ns
8.	37	38	**	ns	ns	ns
9.	17	17	ns	ns	*	ns
10.	20	24	ns	ns	ns	ns
11.	1	9	ns	+8**	ns	ns
12.	4	12	*	ns	ns	ns
13.	21	15	ns	ns	ns	ns
14.	30	30	***	ns	ns	ns
15.	8	16	*	ns	ns	ns
16.	13	22	ns	ns	ns	ns
17.	41	48	**	ns	ns	ns
18.	34	35	ns	ns	ns	ns
19.	13	16	**	ns	*	ns
20.	8	28	ns	+20***	**	ns
21.	1	9	ns	+8*	ns	ns
22.	4	15	ns	+11*	ns	+19*
23.	No data					
24.	8	22	*	+16***	ns	ns

Responses to sulphur:

Site 1 significant at p=0.081

Note: The mean N recoveries are across all rates of N and give a comparison between extra N applied as ammonium nitrate and urea. Absloute values will be higher at low rates of extra N applied.

APPENDIX 4. Trial Series A. Grain SDS (mls)

Summary of responses (significant differences p<0.05).

Site	Control SDS (mls)	Control v all N trts	Effect of N rates	U>AN (all rates)	AN * U interaction (all rates)	Sulphur (mls)
1.	62.0	ns	ns	+2.8***	*	+1.7ns
2.	42.0	-	-	-	-	-
3.	49.7	+4.8***	ns	ns	*	ns
4.	No data					
5.	No data					
6.	51.5	+5.1***	ns	+4.1*	*	ns
7.	51.5	ns	ns	-2.9***	ns	ns
8.	54.8	+3.2***	*	ns	ns	+2.3*
9.	51.5	ns	ns	ns	ns	ns
10.	54.5	ns	ns	ns	ns	ns
11.	57.3	ns	ns	ns	ns	ns
12.	43.8	ns	ns	ns	*	ns
13.	62.5	ns	ns	ns	ns	ns
14.	52.3	ns	**	ns	ns	+1.0*
15.	64.3	ns	ns	ns	ns	+6.7***
16.	58.3	+3.2*	ns	ns	ns	ns
17.	39.0	ns	*	+3.5*	ns	ns
18.	65.0	ns	ns	ns	ns	ns
19.	63.5	ns	ns	+5.6***	ns	ns
20.	72.0	+4.4**	**	+2.6**	ns	ns
21.	44.3	ns	ns	ns	ns	ns
22.	71.3	ns	ns	+6.0***	ns	-3.2ns
23.	No data					
24.	64.0	ns	ns	ns	ns	ns

Responses to sulphur:

Site 1 significant at p=0.052
Site 22 significant at p=0.059

APPENDIX 5. Trial Series A. Hagberg Falling Number (HFN)

Summary of responses (significant differences $p<0.05$).

Site	Control HFN	Control v all N trts	Effect of N rates	U>AN (all rates)	AN * U interaction (all rates)	Sulphur
1.	310	ns	ns	ns	ns	ns
2.	298	ns	–	ns	ns	ns
3.	339	ns	ns	+14*	ns	ns
4.	No data					
5.	No data					
6.	307	ns	*	+27***	**	ns
7.	368	–52**	ns	ns	ns	ns
8.	312	+21**	ns	+21***	ns	ns
9.	309	+15*	*	+13**	ns	ns
10.	370	ns	***	ns	ns	ns
11.	377	ns	ns	ns	ns	ns
12.	320	ns	ns	ns	ns	ns
13.	376	ns	ns	ns	ns	ns
14.	357	ns	***	+12***	ns	ns
15.	358	ns	ns	+16***	ns	–9*
16.	337	+16*	ns	+22***	ns	ns
17.	356	ns	*	ns	*	ns
18.	372	ns	ns	+13**	ns	ns
19.	343	ns	ns	+11*	ns	ns
20.	302	ns	ns	ns	ns	ns
21.	277	ns	ns	ns	ns	ns
22.	355	ns	ns	ns	ns	ns
23.	No data					
24.	350	ns	ns	+26***	*	ns

APPENDIX 6. Trial Series A. Specific weight at 85% DM.

Summary of responses (significant differences $p<0.05$).

Site	Control Sp.wt (kg/hl)	Control v all N trts	Effect of N rates	U>AN (all rates)	AN * U interaction (all rates)	Sulphur (kg/hl)
1.	78.2	ns	***	ns	*	ns
2.	No data					
3.	No data					
4.	No data					
5.	No data					
6.	75.4	*	ns	+1.5***	ns	ns
7.	70.6	ns	ns	ns	ns	ns
8.	79.5	ns	ns	+0.6***	*	ns
9.	80.8	ns	ns	ns	ns	-0.6*
10.	No data					
11.	75.8	ns	ns	ns	ns	ns
12.	80.7	ns	ns	ns	ns	ns
13.	78.5	ns	**	-0.9***	ns	-1.0***
14.	77.2	*	ns	ns	*	+0.4ns
15.	79.7	ns	ns	ns	ns	ns
16.	78.9	ns	ns	+0.6**	ns	ns
17.	79.6	ns	ns	+0.5*	ns	ns
18.	69.7	**	ns	ns	ns	ns
19.	79.4	ns	ns	ns	ns	ns
20.	74.2	**	**	ns	ns	ns
21.	78.6	ns	***	-0.5***	**	-0.4*
22.	79.8	ns	ns	-0.3*	ns	ns
23.	No data					
24.	79.9	ns	*	+0.5*	ns	ns

Responses to sulphur:

Site 14 significant at p=0.089

APPENDIX 7. Trial Series A. Sulphur treatments.

Summary of responses (significant differences $p<0.05$)

Site	Grain analysis %S	N:S	Yield (t/ha)	Grain protein (%)	Grain N recovery (%)	Grain SDS (mls)	Grain sp.wt (kg/hl)	Grain HFN
1	0.15	14.5	+0.13*		+5ns	+1.7ns		
2	0.12	15.8					no data	
3	0.12	17.2					no data	
4	no data		no data	no data	no data	no data	no data	no data
5	no data					no data	no data	no data
6	0.15	12.5						
7	0.16	16.2		−0.55*				
8	0.14	14.7	−0.22***	+0.17*		+2.3*		
9	0.14	16.6					−0.6*	
10	0.15	14.1					no data	
11	0.14	19.5	−0.15ns					
12	0.13	19.8						
13	0.13	18.5	+0.36*	−0.22*			−1.0***	
14	0.12	19.4				+1.0*	+0.4ns	
15	No data					+6.7***		−9*
16	No data			+0.38ns				
17	0.15	14.5						
18	0.12	18.5						
19	No data							
20	0.17	15.7	+0.20*	−0.33*				
21	0.14	17.9	−0.12ns				−0.4*	
22	0.20	13.7			+19*	−3.2ns		
23	no data		no data	no data	no data	no data	no data	no data
24	0.19	13.8						

Where response is ns, see Appendices 1–6 for p significance level.

APPENDIX 8. Trial Series B. Grain yield (t/ha at 85% DM)

Summary of responses (significant differences $p<0.05$)

Site	Control yield (t/ha)	Control v all timings (t/ha)	Urea timings	GS for maximum yield	Sulphur (t/ha)
25	9.34	ns	ns	–	ns
26	6.08	+0.51***	***	43–57	ns
27	7.86	+0.26*	ns	–	ns
28	8.95	ns	ns	–	ns
29	7.28	ns	ns	–	ns
30	8.23	ns	ns	–	ns
31	7.30	ns	***	71	ns
32	8.04	ns	ns	–	ns
33	8.67	ns	ns	–	ns
34	7.58	ns	ns	–	ns
35	8.64	-0.53*	ns	–	ns
36	10.84	ns	ns	–	ns
37	7.57	ns	ns	–	ns
38	8.43	ns	ns	–	ns
39	7.43	+0.17**	**	61	-0.11ns
40	9.76	ns	ns	–	ns
41	7.58	+0.19*	ns	–	ns
42	9.14	+0.14*	***	51–65	ns
43	8.85	ns	ns	–	ns
44	8.70	ns	ns	–	ns
45	6.11	ns	**	52	ns
46	9.88	ns	*	43–65	ns
47	7.72	ns	***	–	+0.07ns

Responses to sulphur:

Site 39 significant at p=0.055
Site 47 significant at p=0.054

Note: Optimum Growth Stages for maximum grain yield have been estimated by visual inspection of the data and are given as a guide only.

APPENDIX 9. Trial Series B. Grain protein (% at 86% DM)

Summary of responses (significant differences $p<0.05$)

Site	Control protein (%)	Control v all timings(%)	Urea timings	GS for maximum protein	Sulphur (%)
25	9.40	+0.80***	***	75	ns
26	6.87	+1.00***	***	43–83	ns
27	8.10	+0.60***	***	77	ns
28	10.70	+0.60***	***	75–80	ns
29	11.35	+0.50**	*	71–75	ns
30	10.63	ns	ns	–	ns
31	12.61	+0.43***	***	53–75	ns
32	10.96	+0.49**	ns	–	ns
33	11.24	+0.43***	ns	–	ns
34	11.75	ns	ns	–	ns
35	11.70	+0.59***	ns	–	ns
36	10.96	ns	ns	–	ns
37	10.78	+0.29*	**	39–45	ns
38	11.95	+0.53***	ns	–	+0.27ns
39	7.45	+0.63***	***	68	ns
40	11.58	ns	*	75	ns
41	10.83	+0.29*	ns	–	ns
42	12.23	+1.53***	***	75	ns
43	12.58	ns	*	59–69	ns
44	10.81	+0.55***	ns	–	ns
45	12.87	ns	ns	–	ns
46	10.05	+0.34*	ns	–	ns
47	12.24	+0.57***	**	73–83	+0.12ns

Responses to sulphur:

Site 38 significant at p=0.063
Site 47 significant at p=0.066

Note: Optimum Growth Stages for maximum grain protein have been estimated by vis inspection of the data and are given as a guide only.

APPENDIX 10. Trial Series B. Apparent recovery of N in grain (%)

Summary of responses (significant differences $p<0.05$)

Site	Mean of all timings	Urea timings	GS for maximum N recovery	Sulphur (%)
25	33	**	75	ns
26	42	***	43–57	ns
27	43	*	77	ns
28	22	***	75	ns
29	22	**	71	ns
30	12	*	65	ns
31	16	**	53–71	ns
32	11	ns	–	ns
33	20	ns	–	ns
34	1	ns	–	ns
35	–7	ns	–	ns
36	–16	ns	–	ns
37	13	*	45–53	ns
38	21	ns	–	+10ns
39	27	***	43–71	ns
40	14	**	75	ns
41	19	ns	–	ns
42	69	***	51–59	ns
43	3	**	59–69	–9*
44	20	*	46	ns
45	–4	*	–	ns
46	17	*	65	ns
47	16	***	59,75–83	+8**

Sulphur responses:

Site 38 significant at p=0.057

Note: Optimum Growth Stages for maximum nitrogen recovery have been estimated b visual inspection of the data and are given as a guide only.

APPENDIX 11. Trial Series B. Grain SDS (mls)

Summary of responses (significant differences $p<0.05$)

Site	Control SDS (mls)	Control v all timings	Urea timings	GS for maximum SDS	Sulphur (mls)
25	No data				
26	45.9	+4.0***	**	75–77	ns
27	49.6	+2.0***	***	63–77	ns
28	56.7	ns	ns	–	ns
29	55.4	ns	*	65–71	ns
30	49.5	ns	ns	–	ns
31	62.1	ns	*	–	ns
32	65.4	ns	ns	–	ns
33	59.1	ns	ns	–	ns
34	50.5	ns	ns	–	ns
35	61.0	ns	ns	–	ns
36	59.3	ns	ns	–	ns
37	68.3	+3.6**	ns	–	ns
38	75.6	ns	ns	–	ns
39	51.8	ns	ns	–	ns
40	51.8	ns	*	39	ns
41	52.5	+1.8*	**	39–75	ns
42	42.1	ns	ns	–	ns
43	67.0	ns	***	39–73	ns
44	60.0	ns	*	71–75	ns
45	72.0	-3.3*	ns	–	ns
46	60.0	+2.4*	ns	–	ns
47	65.0	ns	ns	–	ns

Note: Optimum Growth Stages for maximum grain SDS have been estimated by visual inspection of the data and are given as a guide only.

APPENDIX 12. Trial Series B. Grain Hagberg Falling Number (HFN))

Summary of responses (significant differences $p<0.05$)

Site	Control HFN	Control v all timings	Urea timings	GS for maximum HFN	Sulphur
25	No data				
26	62	+42***	***	75–83	ns
27	295	ns	ns	–	ns
28	303	ns	*	39–75	ns
29	310	ns	ns	–	ns
30	217	+29***	ns	–	ns
31	340	ns	ns	–	+10*
32	378	+11*	ns	–	ns
33	298	ns	ns	–	+15**
34	351	ns	ns	–	ns
35	311	+16***	ns	–	ns
36	320	ns	ns	–	ns
37	335	+12*	*	64–71	–18**
38	399	ns	ns	–	ns
39	348	ns	ns	–	ns
40	356	ns	ns	–	ns
41	343	ns	ns	–	ns
42	279	ns	ns	–	ns
43	342	ns	*	–	ns
44	332	ns	ns	–	ns
45	279	ns	ns	–	ns
46	315	ns	ns	–	ns
47	347	ns	ns	–	ns

Note: Optimum Growth Stages for maximum HFN have been estimated by visual inspection of the data and are given as a guide only.

APPENDIX 13. Trial Series B. Specific weight (kg/hl at 85% DM)))

Summary of responses (significant differences $p<0.05$)

Site	Control (kg/hl)	Control v all timings	Urea timings	GS for maximum Sp.wt.	Sulphur (kg/hl)
25	No data				
26	72.7	+1.1***	***	43–83	ns
27	75.2	+0.9***	***	63–77	ns
28	81.1	+0.4*	ns	–	ns
29	75.7	ns	ns	–	ns
30	No data				
31	81.7	ns	ns	–	ns
32	79.1	ns	ns	–	ns
33	79.7	ns	**	–	–0.2ns
34	76.7	ns	ns	–	ns
35	80.5	ns	ns	–	–1.0*
36	No data				
37	82.6	ns	ns	–	–0.4ns
38	77.2	ns	ns	–	ns
39	83.2	+0.4**	***	43–71	–0.3*
40	No data				
41	78.0	ns	ns	–	ns
42	78.8	ns	ns	–	ns
43	77.5	ns	ns	–	ns
44	81.8	ns	ns	–	ns
45	76.6	ns	ns	–	–0.8*
46	82.4	ns	ns	–	ns
47	79.8	ns	ns	–	ns

Responses to sulphur:

Site 33 significant at p=0.058
Site 37 significant at p=0.054

Note: Optimum Growth Stages for maximum specific weight have been estimated by visual inspection of the data and are given as a guide only.

APPENDIX 14. Trial Series B. Sulphur treatments.

Summary of responses (significant differences $p<0.05$)

Site	Grain analysis %S	N:S	Yield (t/ha)	Grain protein (%)	Grain N recovery (%)	Grain SDS (mls)	Grain sp.wt (kg/hl)	Grain HFN
25	no data					no data	no data	no data
26	0.13	12.9						
27	no data							
28	no data							
29	no data							
30	0.11	21.1					no data	
31	0.15	18.4						+10*
32	0.15	15.4						
33	0.14	16.8					−0.2ns	+15*
34	no data							
35	no data							
36	no data						−1.0*	
						no data		
37	0.15	15.5						−18*
38	0.13	19.6		+0.27ns	+10ns		−0.4ns	
39	0.12	14.1	−0.11ns				−0.3*	
40	0.13	18.8					no data	
41	0.12	19.2						
42	0.20	14.3						
43	0.19	13.9			−9*			
44	0.22	10.8						
45	no data						−0.8*	
46	no data							
47	0.22	11.8	+.07ns	+0.12ns	+8*			

Where response is ns, see Appendices 8–13 for p significance level.

APPENDIX 15a **Trial Series A. Soil and grain sulphur analysis data.**

		Treatment (kg/ha Urea N)											
		30		60		120		30 + S		60 + S		120 + S	
Site	Soil P SO_4-S (mg/l)	%S	N:S	%S	N:S	%S	N:S	%S	N:S	%S	N:S	%S	N:S
1	-	0.15	14.5	0.15	14.9	0.14	17.1	0.14	15.7	0.14	16.1	0.14	16.7
2	-	0.12	15.8	0.12	16.8	0.14	16.6	0.12	16.3	0.13	17.2	0.13	17.9
3	-	0.12	17.2	0.11	20.8	0.12	21.2	0.11	18.5	0.13	17.6	0.12	21.2
4	-	No data											
5	-	No data											
6	-	0.15	12.5	0.15	14.1	0.15	16.4	0.14	13.9	0.15	14.4	0.15	16.2
7	-	0.16	16.2	0.16	16.5	0.16	17.6	0.16	15.1	0.16	16.1	0.16	16.9
8	3.0	0.14	14.7	0.14	15.6	0.15	15.7	0.14	15.0	0.15	14.9	0.14	16.9
9	5.4	0.14	16.6	0.13	18.2	0.13	19.0	0.13	17.7	0.14	17.0	0.14	17.7
10	4.5	0.15	14.1	0.15	15.2	0.15	16.1	0.14	15.6	0.15	15.1	0.15	16.9
11	5.0	0.14	19.5	0.15	18.3	0.16	17.9	0.14	19.5	0.15	19.0	0.16	18.0
12	4.7	0.13	19.8	0.14	18.8	0.13	21.4	0.13	19.5	0.13	20.1	0.14	20.4
13	-	0.13	18.5	0.13	20.0	0.15	18.5	0.15	15.9	0.14	18.2	0.15	18.1
14	3.3	0.12	19.4	0.13	18.5	0.12	22.0	0.13	17.4	0.12	20.8	0.12	22.2
15	<2.5	No data											
16	-	No data											
17	-	0.15	14.5	0.15	15.5	0.15	17.0	0.15	14.3	0.14	16.8	0.14	17.9
18	3.8	0.12	18.5	0.12	18.8	0.14	17.9	0.13	15.9	0.12	18.9	0.14	17.5
19	2.8	-	-	-	-	-	-	0.18	15.5	0.19	14.8	0.19	15.7
20	7.2	0.17	15.7	0.17	16.2	0.17	17.6	0.16	15.6	0.16	17.1	0.18	16.7
21	4.9	0.14	17.9	0.15	17.7	0.14	19.6	0.15	17.1	0.14	18.8	0.14	20.2
22	2.6	0.20	13.7	0.20	14.1	0.19	15.7	0.21	13.2	0.21	13.5	0.21	14.2
23	-	No data											
24	3.2	0.19	13.8	0.18	15.1	0.17	17.5	0.17	15.4	0.19	14.4	0.20	15.0

APPENDIX 156 Trial Series B. Soil and grain sulphur analysis data.

Site	Soil P SO_4-4 (mg/l)	+ 30 days		+ 40 days		+ 50 days		+ 30 days + S		+ 40 days + S		+ 50 days + S	
		%S	N:S	%S	N:S	%S	N:S	%S	N:S	%S	N:S	%S	N:S
25	-	No data											
26	-	0.13	12.9	0.13	12.7	0.13	13.0	0.13	13.2	0.14	12.0	0.13	12.5
27	-	No data											
28	-	No data											
29	-	No data											
30	-	0.13	17.3	0.09	24.9	0.10	21.2	0.18	12.6	0.13	17.5	0.15	14.5
31	-	0.15	18.1	0.15	18.3	0.14	18.8	0.16	17.1	0.14	19.1	0.14	18.8
32	5.0	0.16	14.9	0.15	15.8	0.15	15.4	0.16	15.3	0.15	15.8	0.15	15.1
33	11.8	0.15	15.9	0.14	17.4	0.14	17.2	0.15	16.3	0.15	16.2	0.14	17.0
34	8.0	-	-	-	-	-	-	0.14	18.5	0.15	17.2	0.13	18.9
35	-	No data											
36	3.5	No data											
37	4.3	0.15	15.3	0.15	15.2	0.14	15.9	0.15	15.3	0.15	15.5	0.15	15.5
38	5.5	0.13	19.0	0.14	18.4	0.12	21.3	0.13	19.6	0.14	18.8	0.13	19.9
39	-	0.11	15.7	0.12	13.8	0.12	12.9	0.12	14.3	0.17	9.6	0.12	13.3
40	6.0	0.13	18.3	0.13	18.5	0.13	19.5	0.14	17.4	0.13	18.5	0.14	18.0
41	-	0.12	19.3	0.12	19.4	0.12	18.9	0.13	17.4	0.13	17.7	0.12	19.3
42	-	0.19	14.7	0.20	13.7	0.21	14.4	0.21	13.6	0.20	14.5	0.21	13.9
43	11.8	0.20	13.3	0.18	14.6	0.19	13.8	0.20	12.9	0.19	14.0	0.16	16.3
44		0.21	11.0	0.21	11.2	0.23	10.1	0.22	10.3	0.22	11.0	0.21	11.2
45	3.5												
46	5.8	-	-	-	-	-	-	0.16	13.1	0.17	12.4	0.17	12.6
47	2.7	0.23	11.1	0.21	12.7	0.23	11.5	0.22	11.8	0.21	12.7	0.23	11.7

APPENDIX 16

KNEESALL, 1988

Treatment (kg/ha N)

	0	30	60	90	120	180	LSD	CV
Flour Extraction Rate (%)								
Amm. Nit	75.3	75.2	75.4	75.0	74.9	75.7	0.9	0.9%
Urea		75.2	74.4	74.9	74.6	74.8		
Urea + S		75.0	75.4		74.7			
Flour Colour (GCF Units)								
Amm. Nit	0.8	0.8	1.1	1.6	1.9	1.9	0.7	31.1%
Urea		0.9	2.0	2.0	2.2	2.5		
Urea + S		1.0	1.5		2.5			
Protein Kjeldahl (%)								
Amm. Nit	7.8	8.8	9.1	9.7	10.0	10.2	0.6	4.5%
Urea		8.6	9.9	10.1	10.5	11.3		
Urea + S		8.6	9.4		10.8			
Protein NIR (%)								
Amm. Nit	7.4	8.5	8.9	9.5	9.8	10.0	0.6	4.7%
Urea	8.3	9.6	9.8	10.3	11.1			
Urea + S		8.2	9.2		10.7			
Loaf Volume (ml)								
Amm.Nit	1235	1339	1326	1373	1424	1392	55	4%
Urea		1308	1412	1417	1422	1450		
Urea + S		1337	1399		1470			
Loaf Score (Max 50)								
Amm. Nit	15	23	22	26	30	27	5	18%
Urea		21	30	32	30	36		
Urea + S		21	31		36			

BOYT, 1989

Treatment (Kg/ha N)

	0	30	60	90	120	180	LSD	CV%
Flour Extraction Rate (%)								
Amm. Nit	76.80	76.05	76.27	76.30	76.05	76.47	0.985	0.9%
Urea		76.47	76.17	76.40	75.90	76.27		
Urea + S		75.35	74.90		75.92			
Flour Colour (GCF Units)								
Amm. Nit	-0.82	-1.12	-0.77	-0.86	-0.66	-0.65	0.543	62.0%
Urea		-0.27	-0.86	-0.15	-0.67	-0.07		
Urea + S		-1.00	-0.22		-0.66			
Protein Kjeldahl (%)								
Amm. Nit	9.70	9.52	9.90	10.22	10.35	10.62	0.348	2.4%
Urea		9.70	10.07	10.35	10.62	11.05		
Urea + S		9.75	10.17		10.82			
Protein NIR (%)								
Amm.Nit	8.90	9.10	9.60	9.70	9.90	10.10	0.368	2.6%
Urea		9.27	9.55	9.80	10.22	10.62		
Urea + S		9.20	9.72		10.35			
Loaf Volume (ml)								
Amm.Nit	1347.5	1333.7	1382.5	1377.5	1407.5	1412.5	44.51	2.2%
Urea		1353.7	1397.5	1426.2	1410.0	1485.0		
Urea + S		1366.2	1407.5		1482.5			
Loaf Score (max 50)								
Amm. Nit	21.7	20.0	24.0	27.2	25.7	25.5	3.81	10.3%
Urea		24.2	26.7	28.0	25.7	29.5		
Urea + S		25.2	26.2		32.0			

HARGRAVE, 1989

Treatment (Kg/ha N)

	0	30	60	90	120	180	LSD	CV%
Flour Extraction Rate (%)								
Amm. Nit	78.72	78.85	78.95	79.22	79.12	79.22	0.277	0.2%
Urea		79.18	79.17	79.07	78.47	78.55		
Urea + S		78.85	78.62		78.62			
Flour Colour (GCF Units)								
Amm. Nit	-1.87	-2.27	-1.85	-1.00	-1.05	-0.80	0.411	25.3%
Urea		-1.58	-0.92	-0.74	-0.65	-0.21		
Urea + S		-1.38	-0.87		-0.69			
Protein Kjeldahl (%)								
Amm. Nit	8.50	9.20	9.82	10.40	10.55	10.80	0.317	2.2%
Urea		9.47	10.15	10.22	11.00	11.55		
Urea + S		9.60	10.32		11.05			
Protein NIR (%)								
Amm. Nit	8.12	8.77	9.45	10.00	10.17	10.37	0.184	1.3%
Urea		9.10	9.75	10.15	10.60	11.15		
Urea + S		9.22	9.92		10.77			
Loaf Volume (ml)								
Amm.Nit	1146.2	1191.2	1200.0	1265.0	1263.7	1272.5	45.86	2.6%
Urea		1172.1	1252.5	1238.7	1257.5	1302.5		
Urea + S		1211.2	1252.5		1258.7			
Loaf Score (max 50)								
Amm. Nit	11.7	14.2	14.5	18.5	17.7	19.5	4.40	17.8%
Urea		14.8	18.5	16.2	19.0	19.2		
Urea + S		18.5	19.5		19.2			

FOLKINGHAM, 1989

Treatment (Kg/ha N)

	0	30	60	90	120	180	LSD	CV%
Flour Extraction Rate (%)								
Amm. Nit	76.7	76.82	77.85	77.20	77.27	77.45	0.894	0.8%
Urea		77.37	77.62	76.85	76.85	76.67		
Urea + S		77.12	77.40		76.82			
Flour Colour (GCF Units)								
Amm. Nit	-0.31	-0.20	0.00	0.20	0.00	0.25	0.517	134%
Urea		-0.07	0.37	0.37	0.80	0.97		
Urea + S		0.10	0.27	1.01				
Protein Kjeldahl (%)								
Amm. Nit	9.25	9.75	10.00	10.50	10.60	10.90	0.631	4.2%
Urea		10.00	10.15	10.52	11.22	11.62		
Urea + S		10.20	10.32		11.37			
Protein NIR (%)								
Amm. Nit	9.02	9.57	9.82	10.27	10.42	10.77	0.596	4.1%
Urea		9.85	10.07	10.27	10.95	11.60		
Urea + S		10.00	10.10		11.10			
Loaf Volume (ml)								
Amm. Nit	1227.5	1241.2	1262.5	1262.5	1347.5	1325.0	77.52	4.2%
Urea		1280.0	1320.0	1327.5	1326.2	1352.5		
Urea + S		1290.0	1342.5		1333.7			
Loaf Score (max 50)								
Amm. Nit	13.7	14.7	17.5	17.5	23.2	17.7	5.57	20.6%
Urea		17.0	20.0	20.7	20.5	21.5		
Urea + S		18.5	20.2		21.7			

FRAMPTON, 1990

Treatment (Kg/ha N)

	0	30	60	90	120	180	LSD	CV%
Flour Extraction Rate (%)								
Amm. Nit	76.33	76.58	76.65	76.73	76.68	76.50	0.51	0.5%
Urea		76.35	76.63	76.60	76.58	75.97		
Urea + S		76.60	76.28		76.43			
Flour Colour (GCF Units)								
Amm.Nit	-1.600	-1.725	-1.325	-1.233	-1.050	-0.550	0.497	36.6%
Urea		-1.437	-1.250	-0.587	-0.238	-0.017		
Urea + S		-1.388	-0.775		-0.150			
Protein (Kjeldahl, %)								
Amm. Nit	8.28	8.68	9.48	9.47	9.83	10.30	0.43	3.1%
Urea		8.88	9.48	10.00	10.35	10.93		
Urea + S		9.18	9.80		10.63			
Protein (NIR, %)								
Am. Nit	8.28	8.73	9.35	9.50	9.93	10.28	0.44	3.1%
Urea		8.88	9.43	10.15	10.33	10.97		
Urea + S		9.10	9.80		10.65			
Loaf Volume (ml)								
Amm. Nit	1083.7	1101.0	1174.7	1191.7	1172.2	1192.2	81.9	4.9%
Urea		1104.7	1147.2	1215.7	1259.7	1262.7		
Urea + S		1148.2	1157.2		1249.7			
Loaf Score (Max 50)								
Amm. Nit	5.8	4.5	12.3	14.7	11.0	12.0	7.5	42.7%
Urea		6.0	10.0	16.3	17.8	19.7		
Urea + S		10.8	10.5		18.8			

ROUNDWAY, 1990

Treatment (Kg/ha N)

	0	30	60	90	120	180	LSD	CV%
Flour Extraction Rate (%)								
Amm.Nit	74.43	74.85	74.93	74.83	75.00	75.28	0.95	0.9%
Urea		74.63	74.00	74.53	74.03	73.38		
Urea + S		74.53	74.20		73.98			
Flour Colour (GCF Units)								
Amm. Nit	-1.250	-1.338	-1.012	-0.987	-0.850	-0.600	0.391	35.7%
Urea		-1.212	-0.712	-0.638	-0.338	0.088		
Urea + S		-1.013	-0.650	-0.225				
Protein (Kjel, %)								
Amm. Nit	9.20	9.50	10.10	10.28	10.55	10.83	0.32	2.1%
Urea		9.73	10.08	10.60	11.08	11.83		
Urea + S		9.80	10.25		11.38			
Protein (NIR, %)								
Amm. Nit	9.25	9.50	10.00	10.40	10.50	10.90	0.30	2.0%
Urea		9.78	10.05	10.58	11.15	11.88		
Urea + S		9.95	10.38		11.40			
Loaf Volume (ml)								
Amm. Nit	1131.2	1143.7	1153.7	1152.5	1167.5	1181.2	57.1	3.4%
Urea		1147.5	1155.0	1187.5	1207.5	1211.2		
Urea + S		1167.5	1202.5		1240.0			
Loaf Score (Max 50)								
Amm. Nit	5.5	5.8	6.8	8.8	10.5	10.0	3.9	29.6%
Urea		8.0	8.8	9.3	10.3	11.0		
Urea + S		8.8	11.3		13.0			

BISHOPS CANNING, 1991
Treatment (Kg/ha N)

	0	30	60	90	120	180	LSD	CV%
Flour Yield (%)								
Am. Nit	78.90	79.20	79.03	78.80	78.88	78.93	0.87	0.8%
Urea		79.15	78.68	78.98	78.05	77.73		
Urea + S		79.20	78.68		78.45			
Flour Colour (GCF Units)								
Am. Nit	2.537	3.287	3.213	3.537	3.600	4.363	0.711	13.3%
Urea		3.887	3.487	4.525	4.213	5.475		
Urea + S		2.750	3.287		4.238			
Flour Protein (Kjeldahl, %)								
Amm.Nit	10.50	10.73	11.08	11.28	11.30	11.78	0.50	2.9%
Urea		11.88	12.00	12.68	13.10	14.50		
Urea + S		10.85	11.73		13.13			
Flour Protein (NIR, %)								
Amm.Nit	10.53	10.73	11.05	11.15	11.30	11.65	0.32	1.9%
Urea		11.23	11.70	12.48	12.65	14.10		
Urea + S		10.78	11.53		12.70			
LFP Volume (ml)								
Amm.Nit	1533.5	1539.2	1544.7	1584.2	1552.7	1623.0	78.2	3.4%
Urea		1578.5	1571.0	1676.0	1643.5	1773.5		
Urea + S		1536.0	1584.0		1648.0			
LFP Score								
Amm. Nit	30.3	29.8	29.5	32.0	31.8	33.5	3.8	8.5%
Urea		30.8	31.8	34.3	30.8	33.5		
Urea + S		29.8	30.5		32.8			
CBP Volume (ml)								
Amm.Nit	1508.3	1470.7	1505.8	1520.8	1540.3	1526.3	57.6	2.6%
Urea		1498.0	1487.4	1449.4	1471.4	1498.8		
Urea + S		1504.1	1513.8		1496.3			

MILTON ERNEST, 1991
Treatment (Kg/ha N)

	0	30	60	90	120	180	LSD	CV%
Flour Yield								
Amm.Nit	80.98	81.58	81.25	80.88	81.15	81.25	0.89	0.8%
Urea		80.80	81.03	80.43	80.38	80.00		
Urea + S		80.48	80.60		80.50			
Flour Colour (GCF Units)								
Amm.Nit	0.488	1.425	1.150	1.050	1.288	1.288	0.644	31.2%
Urea		0.788	1.362	1.913	2.000	3.075		
Urea + S		1.062	1.125		2.200			
Flour Protein (Kjel, %)								
Amm.Nit	10.60	11.25	11.55	12.10	11.85	11.60	0.46	2.6%
Urea		11.38	12.05	12.83	13.30	14.40		
Urea + S		11.50	12.05		13.48			
Flour Protein (NIR, %)								
Amm.Nit	10.50	11.03	11.33	11.50	11.73	11.50	0.42	2.4%
Urea		11.18	11.70	12.30	13.13	13.80		
Urea + S		11.40	11.83		13.13			
LFP Volume (ml)								
Amm. Nit	1492.5	1546.2	1511.2	1562.5	1585.0	1527.5	79.4	3.6%
Urea		1472.5	1533.7	1593.7	1630.0	1617.5		
Urea + S		1520.0	1607.5		1635.0			
LFP Score								
Amm.Nit	26.8	30.8	30.3	31.5	32.8	31.5	4.5	10.0%
Urea		27.5	32.0	32.8	34.8	33.0		
Urea + S		30.5	34.8		35.3			
CBP Volume (ml)								
Amm. Nit	1599.5	1584.5	1590.5	1607.0	1595.5	1600.0	43.5	1.9%
Urea		1599.7	1620.2	1611.7	1614.0	1589.2		
Urea + S		1569.7	1598.0		1622.7			
Wet Gluten (%)								
Amm. Nit	30.75	33.15	32.35	34.40	34.15	34.20	4.22	5.5%
Urea		36.70	35.20	37.35	37.35	41.25		
Urea + S		35.10	35.25		39.75			
Gluten Index								
Amm.Nit	63.4	61.4	56.1	59.5	57.4	44.7	17.03	14.3%
Urea		52.9	63.0	53.7	56.5	55.0		
Urea + S		49.8	46.3		52.3			

APPENDIX 17

DETLING, 1988

Growth Stage

	Basal	Am. Nitrate	GS39	GS	GS61	GS65	GS67	GS69	LSD	CV
Flour Extraction Rate (%)										
Control	74.2	74.2							0.7	0.7%
Urea			74.3	73.8	74	74.2	73.6	74		
Urea + S						73.4	74	74.2		
Flour Colour (GCF Units)										
Control	0.1	0.9							0.4	37.1%
Urea			0.9	1.1	0.9	0.8	1.1	0.5		
Urea + S						1.3	1.0	0.9		
Protein Kjeldahl (%)										
Control	7.9	9.8							0.4	3.5%
Urea			10.1	10.2	10.2	10.2	10.2	9.7		
Urea + S						10.2	10.0	9.9		
Protein NIR (%)										
Control	7.4	9.6							0.4	3.4%
Urea			9.9	10.0	10.1	10.0	10.1	9.5		
Urea + S						10.1	10.1	9.6		
Loaf Volume (ml)										
Control	1350	1435							65	3.7%
Urea			1445	1450	1462	1479	1462	1432		
Urea + S						1412	1461	1446		
Loaf Score (Max 50)										
Control	16	31							5	12.6%
Urea			31	33	32	34	32	30		
Urea + S						31	31	31		

BETHERSDEN, 1989

	Growth Stage											
	Nil	**Nil**	**Nil**	**GS39**	**GS56**	**GS60/61**	**GS69**	**GS78**	**GS83**	**GS85/86**	**LSD**	**CV%**
Flour Extraction Rate (%)												
Control	75.15	75.05	75.02									
Urea				75.00	75.00	74.97	74.85	75.07	75.57	75.05	0.336	0.3%
Urea + S							74.47	75.00	75.07			
Flour Colour (GCF Units)												
Control	-0.65	-0.60	-0.35									
Urea				-0.67	-0.81	-0.47	-0.19	-0.47	-0.30	-0.47	0.378	57.3%
Urea + S						-0.04	-0.30	-0.65				
Protein Kjeldahl (%)												
Control	10.05	10.07	10.20									
Urea				10.65	10.55	10.55	11.07	10.75	10.50	10.35	0.348	2.3%
Urea + S							11.22	10.82	10.52			
Protein NIR (%)												
Control	9.80	9.85	9.92									
Urea				10.45	10.32	10.30	10.75	10.52	10.35	10.07	0.301	2.0%
Urea + S							11.00	10.77	10.22			
Loaf volume (ml)												
Control	1436.2	1441.2	1502.5									
Urea				1475.0	1468.7	1498.7	1518.7	1491.2	1501.2	1437.5	63.29	3.0%
Urea + S							1496.2	1463.7	1453.7			
Loaf Score (max 50)												
Control	30.2	30.0	32.75									
Urea				32.0	32.0	32.7	35.2	31.0	31.0	29.0	4.51	10.0%
Urea + S							32.5	29.7	29.5			

MORLEY, 1989

	Growth Stage											
	Nil	**Nil**	**GS41**	**GS51**	**GS71**	**GS75**	**GS77**	**GS83**	**GS87**	**GS93**	**LSD**	**CV%**
Flour Extraction Rate (%)												
Control	78.62	78.32										
Urea			77.92	78.19	78.85	78.55	77.95	78.27	78.60	78.60	0.726	0.6%
Urea + S						77.94	78.05	78.52				
Flour Colour (GCF Units)												
Control	0.05	-0.40										
Urea			0.15	0.26	0.06	0.60	0.20	-0.11	0.20	0.02	0.528	268%
Urea + S						0.37	0.10	0.27				
Protein Kjeldahl (%)												
Control	10.97	10.90										
Urea			11.25	11.72	11.50	11.67	11.50	11.15	10.90	10.77	0.356	2.2%
Urea + S						11.72	11.45	10.95				
Protein (NIR) (%)												
Control	10.92	10.75										
Urea			11.30	11.73	11.57	11.62	11.37	11.30	11.05	10.77	0.330	2.0%
Urea + S						11.77	11.47	10.95				
Loaf volume (ml)												
Control	1470.0	1491.2										
Urea			1505.0	1493.8	1543.7	1510.0	1541.2	1442.5	1497.5	1511.2	42.67	2.0%
Urea + S						1541.2	1517.5	1461.2				
Loaf Score (max 50)												
Control	27.7	30.0										
Urea			30.2	29.5	31.2	31.0	32.0	25.2	29.0	28.5	3.690	8.8%
Urea + S						30.7	31.2	25.0				

TERRINGTON, 1989

	Growth Stage											
	Nil	**Nil**	**GS39**	**GS50**	**GS61**	**GS71**	**GS77**	**GS83**	**GS87**	**GS92**	**LSD**	**CV%**
Flour Extraction Rate (%)												
Control	77.77	77.72										
Urea			77.70	77.40	77.50	77.60	77.37	77.80	77.85	77.90	0.380	0.3%
Urea + S						77.15	77.10	77.75				
Flour Colour (GCF Units)												
Control	0.74	0.57										
Urea			0.55	0.84	0.62	0.62	0.67	0.90	0.71	0.60	0.296	28.3%
Urea + S						0.85	0.97	0.84				
Protein Kjeldahl (%)												
Control	10.67	10.52										
Urea			10.67	10.90	10.87	10.75	10.92	10.92	10.85	10.50	0.198	1.3%
Urea + S						10.90	11.00	10.82				
Protein NIR (%)												
Control	10.27	10.20										
Urea			10.40	10.52	10.65	10.37	10.65	10.65	10.60	10.15	0.238	1.6%
Urea + S						10.62	10.72	10.57				
Loaf Volume (ml)												
Control	1313.7	1350.0										
Urea			1367.5	1315.0	1362.5	1353.7	1403.7	1343.7	1351.2	1371.2	47.74	2.5%
Urea + S						1346.2	1352.5	1320.0				
Loaf Score (max 50)												
Control	19.5	23.0										
Urea			21.0	19.5	21.0	21.5	24.0	22.5	19.7	21.7	4.78	15.7%
Urea + S						21.5	21.2	19.2				

BETHERSDEN, 1990

	Growth Stage											
	Nil	**Nil**	**GS39**	**GS41**	**GS58**	**GS64**	**GS69**	**GS75**	**GS83**	**GS86**	**LSD**	**CV%**
Flour Extraction Rate (%)												
Control	75.55	75.53									0.54	0.5
Urea			75.30	75.45	75.30	75.33	75.35	75.03	75.77	75.83		
Urea + S						75.15	74.83	75.33				
Flour Colour (GCF Units)												
Control	-1.987	-1.950									0.23	9.0
Urea			-1.875	-1.687	-1.700	-1.775	-1.638	-1.475	-1.732	-1.900		
Urea + S						-1.812	-1.950	-1.550				
Protein (Kjeldahl, %)												
Control	10.23	10.20									0.55	3.7
Urea			10.48	10.63	10.55	10.45	10.63	10.88	10.58	10.53		
Urea + S						10.48	10.53	10.93				
Protein (NIR, %)												
Control	10.18	10.15									0.29	2.0
Urea			10.38	10.53	10.55	10.33	10.58	10.75	10.32	10.03		
Urea + S						10.48	10.43	10.80				
Loav Volume (ml)												
Control	1193.5	1187.2									41.9	2.4
Urea			1213.0	1248.0	1206.0	1231.0	1258.0	1227.2	1246.8	1192.2		
Urea + S						1249.2	1207.2	1291.7				
Loaf Score (max 50)												
Control	13.0	14.0									4.5	21.0
Urea			14.3	14.5	12.5	14.8	17.5	16.0	12.8	13.8		
Urea + S						16.0	15.0	19.8				

SUTTERBY, 1990

	Growth Stage											
	Nil	**Nil**	**GS39-41**	**GS45**	**GS59**	**GS65**	**GS68**	**GS75**	**GS83**	**GS85**	**LSD**	**CV%**
Flour Extraction Rate (%)												
Control	76.60	76.60									0.54	0.5
Urea			77.03	76.90	76.95	76.58	76.70	76.65	76.60	76.85		
Urea + S						76.85	76.40	76.38				
Flour Colour (GCF Units)												
Control	-0.775	-0.700										
Urea			-0.775	-0.550	-0.625	-0.775	-0.550	-0.550	-0.650	-0.863	0.36	38.4%
Urea + S						-0.675	-0.475	-0.625				
Protein (Kjel, %)												
Control	8.83	8.78									0.43	3.3%
Urea			9.08	9.39	9.33	9.25	9.50	9.03	9.23	8.58		
Urea + S						9.05	9.53	9.50				
Protein (NIR, %)												
Control	8.63	8.58									0.49	3.8
Urea			8.93	9.15	9.18	9.03	9.28	8.80	8.98	8.40		
Urea + S						8.95	9.28	9.25				
Loaf Volume (ml)												
Control	1195.0	1198.7									75.2	4.4
Urea			1110.0	1213.7	1163.7	1211.2	1203.7	1215.0	1218.7	1132.5		
Urea + S						1188.7	1211.2	1216.2				
Loaf Score (max 50)												
Control	15.3	14.5									3.8	17.9
Urea			14.8	16.0	12.3	15.3	15.5	16.8	15.8	12.5		
Urea + S						14.0	15.8	14.5				

TERRINGTON, 1990

	Growth Stage											
	Nil	**Nil**	**GS39**	**GS49**	**GS59**	**GS65**	**GS71**	**GS75**	**GS79**	**GS86**	**LSD**	**CV%**
Flour Extraction Rate (%)												
Control	76.60	77.07									1.04	0.9
Urea			77.25	77.22	76.60	76.12	76.70	76.65	77.05	76.90		
Urea + S					76.55	77.05	76.15					
Flour Colour (GCF Units)												
Control	-1.04	-0.20									1.42	735.1
Urea			0.34	-0.57	0.32	1.06	-0.79	-0.21	-0.84	-0.10		
Urea + S						-0.04	-0.44	0.75				
Protein, Kjeldahl, %)												
Control	10.58	10.83									0.48	3.0
Urea			11.38	11.43	11.38	11.33	11.48	11.43	11.43	10.60		
Urea + S						11.30	11.50	11.45				
Protein (NIR, %)												
Control	10.43	10.73									0.34	2.1
Urea			11.18	11.28	11.23	11.23	11.35	11.20	11.35	10.75		
Urea + S						11.15	11.38	11.30				
Loaf Volume (ml)												
Control	1440.0	1421.2									48.9	2.3
Urea			1490.0	1477.5	1468.2	1465.0	1485.0	1487.5	1468.7	1471.2		
Urea + S						1440.0	1458.5	1463.5				
Loaf Score (max 50)												
Control	28.8	29.3									3.1	6.9
Urea			33.5	32.3	31.0	29.3	33.0	32.0	32.3	28.8		
Urea + S						30.5	31.5	32.0				

BOXWORTH, 1991

Treatment (Kg/ha N)

Growth Stage

Treatment	Nil	Nil GS75	GS39 GS85	GS49 GS91	GS59 GS67+s	GS67 GS71+s	GS71 GS75+s	LSD	CV%
Flour Yield	81.28	81.30 81.18	81.50 81.20	81.12 81.53	81.70 81.10	81.22 81.28	81.43 81.00	1.04	0.9
Flour Colour (GCF Units)	0.425	0.575 1.100	1.000 1.000	0.625 0.663	0.975 0.412	1.350 1.062	1.125 0.975	0.679	54.5
Flour Protein (Kjel, %)	10.23	10.35 10.93	10.83 11.00	10.70 10.40	10.80 10.58	10.88 11.10	11.10 10.98	0.29	1.8
Flour Protein (NIR, %)	10.00	9.85 10.55	10.48 10.60	10.33 10.08	10.50 10.28	10.43 10.70	10.75 10.63	0.29	1.9
LFP Volume (ml)	1377.5	1380.0 1423.7	1455.0 1398.7	1428.7 1437.5	1411.2 1411.2	1407.5 1485.0	1487.5 1442.5	67.4	3.3
LFP Score (Max 50)	21.8	21.0 24.8	25.5 22.0	25.0 24.0	25.0 24.5	23.3 29.3	28.8 25.3	6.1	17.4
Wet Gluten (%)	28.30	29.05 31.80	32.45 30.10	31.90 31.50	31.35 29.75	30.45 32.25	31.85 32.15	3.11	4.6
Gluten Index	49.5	51.0 56.0	44.5 60.0	61.7 52.0	47.3 38.2	47.4 59.4	46.9 48.9	19.9	17.9

MILTON ERNEST, 1991

Treatment (kg/ha N)

Treatment	Nil	Nil GS75	GS39 GS83	GS45 GS85	GS59 GS69+s	GS69 GS73+s	GS73 GS75+s	LSD	CV%
		Growth Stage							
Flour Yield (%)	81.20	81.33 81.10	81.35 80.60	81.45 81.30	81.13 80.75	80.95 80.88	81.05 80.75	0.54	0.5
Flour Colour (GCF Units)	1.288	1.350 1.675	1.725 1.100	1.587 1.475	1.312 1.352	1.400 1.550	1.550 1.525	0.446	21.4
Flour Protein (Kjel, %)	10.90	10.93 11.78	11.60 11.65	11.40 11.43	11.58 11.43	11,.40 11.90	11.85 11.78	0.25	1.5
Flour Protein (NIR, %)	10.83	10.98 11.68	11.60 11.55	11.38 11.45	11.43 11.43	11.40 11.75	11.93 11.90	0.28	1.7
LFP Volume (ml)	1498.7 1608.7	1502.5 1506.2	1532.5 1537.5	1542.5 1506.2	1583.7 1593.7	1538.7 1570.0	1540.0	74.1	3.3
LFP Score (Max 50)	27.8	27.8 35.8	30.8 25.8	31.0 28.0	31.5 29.3	33.0 35.8	30.8 32.0	4.4	10.1
Wet Gluten (%)	31.10	31.15 34.95	33.90 32.45	32.55 32.35	33.20 31.90	332.50 34.20	33.80 33.75	2.25	3.1
Gluten Index	47.7	55.7 42.1	41.8 50.2	51.7 61.7	49.0 43.9	54.9 48.2	52.5 52.6	17.9	16.4