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The drawing on the cover is from Kjell Aukrust's *«Guttene på broen»*.

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THE COMPOSITION OF HIGH MOLECULAR WEIGHT GLUTENIN SUBUNITS IN NORWEGIAN WHEATS AND THEIR RELATION TO BREAD-MAKING QUALITY

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The high molecular weight (HMW) glutenin subunit composition of Norwegian wheat varieties and advanced breeding lines was determined using polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). The subunit composition was found to vary little between the commercial varieties. Among the breeding lines, however, a more extensive variation was found, comprising three alleles at the Glu-A1 locus, seven alleles at the GLU-B1 locus and two alleles at the GLU-D1 locus. HMW glutenin subunits 13 + 16, reported to be rare in a world collection of wheat varieties, were found to be common in this material. Consistent relationships were found between specific HMW glutenin subunits and bread-making quality, measured by Zeleny sedimentation volume and resistance and extensibility of the extensogram. Subunits 5 + 10 and 13 + 16 had the most positive associations to quality and subunits 2 + 12 the most negative. Approximately 25-27% of the variance in Zeleny sedimentation volume was predicted from the composition of HMW glutenin subunits in this material. It is concluded that SDS-PAGE can be useful as a screening test for bread-making quality in the Norwegian wheat breeding programme.

Key words: Bread-making quality, glutenins, SDS-PAGE, *Triticum aestivum*, wheat.

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The storage proteins of hexaploid wheat are primarily responsible for the unique cohesive-elastic properties of doughs made from wheat flours. These properties are essential in the processing of leavened bread. The storage proteins make up about 85% of the endosperm proteins in the wheat kernels, and they are traditionally divided into the two fractions, gliadin and glutenin, in accordance with their solubility in aqueous alcohol and dilute acid or alkali, respectively (Osborne 1907). Today, these two fractions are usually defined by their state of aggregation in dissociating solutions. Classified like this, the gliadin fraction consists of a complex mixture of single polypeptides, whereas the glutenins consist of polypeptides which are aggregated, mainly by disulphide bonds, to form

large proteins. The different polypeptides that comprise the glutenins are referred to as subunits. These glutenin subunits are further subdivided according to their molecular weight into the high molecular weight (HMW) and the low molecular weight (LMW) subunits of glutenin (Payne et al. 1981b).

The storage proteins of wheat contain about 10% HMW subunits of glutenin, about 40% LMW subunits of glutenin and about 50% gliadins (Payne et al. 1984). Extensive allelic variation in both gliadin and glutenin subunits are detected, giving rise to different compositions of storage proteins between genotypes. This variation causes differences in protein quality between varieties. Protein quality and protein content are the main factors determining bread-making quality.

It is generally believed that wheat varieties of good bread-making quality have larger amounts of the more insoluble storage proteins than those of poor bread-making quality (Pomeranz 1965; Orth & Bushuk 1972; Mecham et al. 1972; Huebner & Wall 1976). Protein quality has therefore been linked with the glutenin group of proteins, which are reported as confering the elastic properties to the dough (Wall 1979). Using gel electrophoresis of reduced glutenin it is possible to study the subunit composition, and to search for differences between wheat varieties related to the protein quality.

The HMW subunits of glutenin are easily resolved by one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), as they are of higher molecular weight than any other major protein subunit in the wheat endosperm. Their molecular weights have been estimated to 95.000-140.000 daltons using SDS-PAGE (Payne et al. 1980). On the polyacrylamide gel they are identified as the 3-5 bands of slowest mobility.

The genes encoding the HMW subunits of glutenin are mapped on the wheat chromosomes. Three loci, Glu-A1, Glu-B1 and Glu-D1, collectively termed the Glu-1 loci, are positioned close to the centromere on the long arm of the homeologous chromosomes 1A, 1B, and 1D, and encode for these subunits (Bietz et al. 1975; Lawrence & Shepherd 1980; Payne et al. 1980; Lawrence & Shepherd 1981). The allelic variation found in these loci in a world collection of 300 hexaploid wheat varieties, analysed and classified by Payne & Lawrence (1983), is shown in Fig. 1. This variation comprises three alleles at the Glu-A1 locus, encoding either a single subunit or no subunit, eleven alleles at the Glu-B1 locus, encoding either one or two subunits, and six alleles at the Glu-D1 locus, encoding two subunits. Lawrence (1986) identified another Glu-B1 allele (subunits 23+24), and another Glu-A1 allele (subunit 25) was found by Sontag et al. (1986).

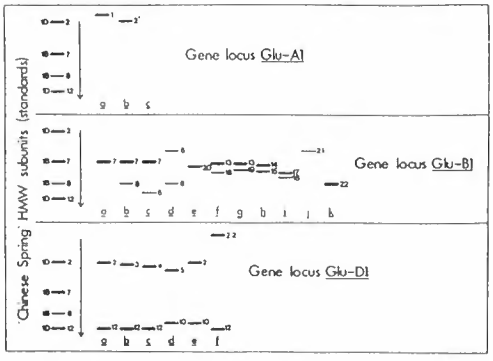

Fig. 1. Allelic variation in the HMW subunits of glutenin. The subunits present in the variety Chinese Spring are given on the left-hand side. The subunits are split into three groups in accordance with whether their genes are controlled by chromosomes 1A, 1B or 1D. The HMW glutenin subunits are denoted by numbers and the alleles by letters (Payne & Lawrence 1983)

Some of the HMW subunits of glutenin were present in very low frequencies in the variety collections analysed. These subunits are presumed to be rare in the

world varieties of hexaploid wheat. Among the rare alleles are the Glu-1B encoded subunits 13+16, 13+19, 14+15, 21 and 22, and the Glu-D1 encoded subunits 2+10 and 2.2+12.

The two HMW subunits of glutenin encoded by Glu-B1 and Glu-D1 are further subgrouped into «x»- and «y»-subunits, the x-subunits being those of slowest mobility on the SDS-polyacrylamide gel (Payne et al. 1981b). The single 1A-subunits are of the x-type. The x-subunits are reported to have about half the cysteine content of the y-subunits (Shewry et al. 1984; Moonen et al. 1985). Marked differences in the nucleotide sequences have also been found between these two subunit types (Halford et al. 1987; Anderson et al. 1988). A third group of HMW subunits of glutenin encoded by the 1B chromosome, the minor 1B z-subunits, is also reported (Holt et al. 1981). This type of subunit seems to be a post-translationally modified form of the major 1B y-subunits.

Only two genes per Glu-1 locus seem to be present, encoding one x-subunit and one y-subunit (Harberd et al. 1986). DNA-sequences encoding a y-subunit have been detected in the 1A- chromosome too, although no 1A y-subunit is produced (Thompson et al. 1983; Forde et al. 1985). 1A y-subunits have never been observed in hexaploid wheat, but they have been found in some wild diploid species related to the progenitor of the A-genome (Waines & Payne 1987). The unexpressed 1A x-subunit of the Glu-A1 c allele, referred to as the null-allele, is another example of such non-functional genes (pseudogenes) among the storage protein alleles (Payne 1987).

Recently, complete DNA-sequences of several of the alleles for HMW subunits of glutenin have been published (Forde et al. 1985; Sugiyama et al. 1985; Halford et al. 1987; Goldsbrough et al. 1988; Anderson et al. 1988). These results reveal that the DNA-sequences of the HMW glutenin subunits can be divided into three structural domains: a non-repetitive N-terminal domain, a repetitive central domain, and a non-repetitive C-terminal domain. The secondary structure of these subunits, predicted on the basis of their DNA-sequences, is reported to be rather unusual with α-helix structure only in the relative hydrophilic N- and C-terminal domains, and regular repetitive β-turns in the central domain (Tatham et al. 1984, 1985; Greene et al. 1988). The presence of the cysteine residues mainly at the N- and C-terminal domains, which are probably exposed to the surface of the protein because of their hydrophilic properties, may facilitate formation of intermolecular disulphide bonds and the formation of linear head-to-tail polymers of the HMW subunits of glutenin (Shewry et al. 1984). Thus, the predicted structure of the HMW subunits of glutenin supports the «linear glutenin hypothesis» proposed by Ewart (1977, 1979).

A number of studies have been carried out relating the individual HMW subunits of glutenin to bread-making quality. Subunit 1 was first reported to be associated with good bread-making quality in contrast to the null-allele when UK-grown varieties were analysed (Payne et al. 1979). The authors suggest that this result is due to either a unique structure of subunit 1, which enables larger and more stable glutenin aggregates to be formed, or to an increased total amount of HMW subunits of glutenin produced in the varieties containing the subunit 1 in contrast to those having the null-allele. Later, subunits 5+10 were related to good bread-making quality in contrast to their allelic counterparts 2+12 (Payne et al. 1981a). This was revealed by analysing progeny from crosses segregating for these subunits. Slight differences in the amino acid sequences between the allelic subunits 5+10 and 2+12 were suggested as an explanation, causing significant differences in their secondary structures. Furthermore, Glu-B1 encoded subunits 13+16 were found to provide better quality than subunits

6+8 on the basis of analyses of progeny from one cross that were segregating in these two alleles (Payne et al. 1981a).

Several other studies analysing other sets of wheats have confirmed that HMW glutenin subunits 5+10 are related to good bread-making quality (Burnouf & Bouriquet 1980; Moonen et al. 1982; Payne et al. 1984; Branlard & Dardevet 1985; Campbell et al. 1987; Cressey et al. 1987; Ng & Bushuk 1988). By analysing random progeny from various crosses and by using isogeneic lines differing in the HMW glutenin subunit alleles, Payne et al. (1984) were able to rank several of the HMW subunits of glutenin according to their effect on the SDS-sedimentation volumes. The following ranking of the Glu-1 alleles was obtained from these results:

Glu-A1: 2* = 1 > 0
Glu-B1: 17+18 = 7+8 > 7+9 > 6+8 > 7
Glu-D1: 5+10 > 2+12 > 3+12 = 4+12
——————————> «Poor quality»

A Glu-1 quality score system was worked out on the basis of these results. This score system enables a quality score (ranging from 3 to 10, where 10 is the best) to be estimated for each variety in terms of the HMW glutenin subunit composition.

Although the HMW subunits of glutenin constitute only about 10% of the storage proteins in the endosperm, this fraction seems to have a major effect on the bread-making quality. As much as 47-60% of the variation in quality was explained by the HMW glutenin subunit composition when 84 UK-grown varieties were analysed (Payne et al. 1987a). Estimates of similar values were obtained when varieties from Spain were analysed (Payne et al. 1988).

Attempts have been made to explore the molecular basis for the verified effects on the bread-making quality of specific HMW glutenin subunits. The better quality found in varieties containing subunits 5+10, in contrast to those with subunits 2+12, has recently been reported as being caused by differences in the y-type subunits 10 and 12 (Payne pers. comm.; Pogna et al. 1987). Studies of the nucleotide sequences of these two subunits have revealed that the better quality conferred by subunit 10 may be due to a more regular β-turn conformation in the central domain providing better elastic properties of this subunit (Goldsbrough et al. 1988). By comparing the nucleotide sequences of the 1Dx subunits 5 and 2, however, Greene et al. (1988) found that subunit 5 contained an additional cysteine residue in the N-terminal region. This additional cysteine residue may affect the covalent cross-linking patterns of the gluten structure, contributing increased strength and superior performance of the dough.

The verified associations between specific HMW subunits of glutenin and bread-making quality are today utilized by wheat breeders, using SDS-PAGE as a screening test for bread-making quality. As this electrophoretic technique is fairly rapid, and can be used on a half grain, selection can be carried out at an early stage in the breeding programme. However, several of the Glu-B1 alleles and a few of the Glu-D1 alleles found in hexaploid wheat have not been tested for their effects on bread-making quality. New alleles have been found in land races of primitive agricultural systems and from diploid and tetraploid wild relatives of common wheats (Law & Payne 1983; Nevo & Payne 1987; Waines & Payne 1987). These represent a gene source that may be exploited further in the quality breeding of wheat.

In the Norwegian wheat breeding programme, high priority is given to improving the bread-making quality. This study was set up in order to examine the composition of HMW glutenin subunits in Norwegian varieties and in adopted breeding material, and to relate the HMW subunits of glutenin present to bread-making quality. An attempt is made to estimate their overall importance regarding the quality in this material.

MATERIALS AND METHODS

The plant materials

A collection of 13 Norwegian spring wheat varieties and 252 advanced spring wheat breeding lines were used to investigate the HMW glutenin subunit compositions in Norwegian wheat materials. The 13 varieties were grown in 1984 at the Department of Crop Science, Agricultural University of Norway, Ås, and their names, pedigree and year of release are given in Table 1. The 252 breeding lines were part of the Norwegian Spring Wheat Breeding Programme in the period 1984-1986. They comprise the selected lines for preliminary and main yield trials in 1984, 1985 and 1986. The main yield trials consisted of 25 lines each year, while the preliminary yield trials consisted of 64 lines in 1984 and 1985, and 49 lines in 1986. To investigate the effect of specific HMW glutenin subunits on bread-making quality, the lines from the preliminary yield trials were used, as these lines had not been subjected to any selection for bread-making quality.

Table 1. Norwegian spring wheat varieties analysed. Their pedigree and the year of variety release are given

Variety	Pedigree	Year of release
Børsum	Land race	
Ås	Selection from a land race	1926
Frøya	Selection from Børsum	1933
Fram I	Jo3/M007	1936
Fram II	Jo3/M007	1940
Snøgg II	Jo3/Sibirian//Ås	1940
Trym	Fylgia/Huron	1951
Norrøna	Fram II/Sopu	1952
Nora	Fram II/Sopu	1959
Rollo	Kärn II/Norrøna	1963
Møystad	Mø 043-40/Kärn II	1966
Runar	Els/7*Rollo	1972
Reno	Tammi/Kärn II//Els	1975

Protein and gluten contents

Protein content was determined by NIR (Ringlund 1978) on wholemeal flour obtained by Falling Number's Laboratory mill 3100, and is presented on a dry weight basis.

Gluten content was determined on white flour milled on a Brabender Quadromat Junior which gave extraction rates of 55 to 65%. Gluten washing was carried out by Falling Number's Glutamatic using a solution of 2% NaCl.

Zeleny sedimentation test

White flour, milled as previously described for measurements of gluten content, was used for the Zeleny sedimentation test. The test was performed according to the procedure of AACC (1983) in which a final solution of 3.5% lactic acid and 17.5% isopropanol is used. The sedimentation volumes were recorded after five minutes resting. In order to obtain a quality parameter independent of the protein content, specific Zeleny sedimentation volumes were calculated. As the relationship between Zeleny sedimentation volume and the protein content was found to be strongly linear in the material analysed, specific Zeleny sedimentation volume was calculated by adjusting the sedimentation volumes to a constant protein level according to the linear regression line. A constant protein level of 12.5% was chosen for these adjustments. As the regression line varied in different years, specific Zeleny sedimentation volume was calculated within years.

Rheological tests and baking

Extensograms and baking tests, carried out using standard methods at the Swedish Cereal Laboratory, Svalöv, were used for studying the rheological properties of doughs and their behaviour during baking. Extensograms were obtained for lines at the preliminary yield trial in 1985 using Brabender's Extensigraph. Resting times of 45 and 90 minutes were used, producing highly corre-

lated results, and therefore only the results from 45 minutes resting are presented in the text. Baking tests were carried out for lines at the preliminary yield trial in 1984.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Total seed protein was extracted using the method of Payne et al. (1980) with some modifications. 0.5 ml sample-buffer of 0.063M Tris-HCl (pH = 6.8) containing 2% SDS, 5% dithiothreitol, 20% glycerol and 0.001% Pyronin Y were added to samples of 20 mg wholemeal flour. Polyacrylamide gels (10%) were cast by the method of Payne et al. (1980), with a 4% stacking gel on top. The electrophoresis was carried out using the Pharmacia Gel Electrophoresis Apparatus GE-2/4 LS, which has a water cooling system and can be suited to gel slabs of 140*180*1.5 mm in size. 15 µl of each sample were loaded on the gel which has the capacity for 14 samples each. The electrophoresis was carried out at 20 mA/gel for the first 15 minutes, and then at 38 mA/gel for about 6 hours until 45 minutes after the tracking dye (Pyronin Y) had eluted from the bottom of the gel. The gels were stained in Coomassie Brilliant Blue R-250 (using a solution of 0.02% Coomassie blue, 5% ethanol and 6% TCA) for at least two days, and then destained in distilled water. To render the gels permanent, they were wrapped in cellophane after being exposed to a solution of 55% methanol and 4% glycerol for 30 minutes, then stretched over a box and dried in air.

The HMW glutenin subunits were classified according to the numbering system of Payne & Lawrence (1983). As described by Payne et al. (1987a), 10% SDS-PAGE cannot separate subunits 2 and 2* adequatly. In some separations, however, these subunits were distinguishable. In order to separate these subunits, thereby distinguishing between varieties with either subunit 2* or the null-allele in addition to subunits 2+12, 5% SDS-PAGE, prepared as described above but with a lower acrylamide concentration, was carried out.

Statistical analyses

The information on the HMW glutenin subunit alleles of the varieties was transferred to 0-1 variables by inserting the value «1» when an allele was present and «0» when an allele was absent. One variable was used for each allele of HMW subunits of glutenin present in the material, which gave a total of 11 variables expressing the HMW subunit composition.

Data were analysed statistically by analysis of variance, t-tests and by stepwise multiple linear regression using the software package MSTAT (Nissen & Mosleth 1986). In addition, a multivariate data analysis, Partial Least Squares (PLS) regression (Wold et al. 1982; Martens 1985) was used. This analysis, which is described in more detail by Mosleth & Uhlen (1990), allows several x-variables to be used simultaneously to describe the variation in one or several y-variables.

In both the multiple regression analysis and the PLS regression analysis, the HMW glutenin subunit composition, expressed as the 11 variables, and the protein content were used as the x-variables. The Zeleny sedimentation volume and also the specific Zeleny sedimentation volume were used as the y-variables. When data from all three years were combined and analysed, another variable expressing the «year of cultivation» (given by the values «1», «2» and «3» for the three years, respectively) was included as an x-variable in the multiple regression analyses and as a second y-variable in the PLS regression analyses. For the PLS regression analyses, the established calibration model is evaluated by «cross-validation» (Wold 1978).

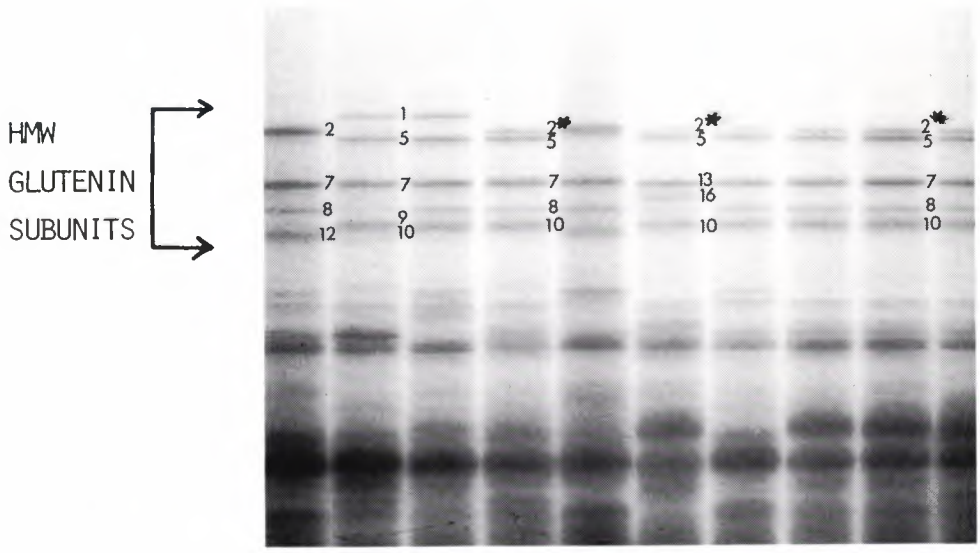

Fig. 2. Electrophoretic patterns of 10 spring wheat breeding lines by using 10% SDS-PAGE. The region of the gel which contains the HMW subunits of glutenin is marked. The subunits have been numbered according to the nomenclature of Payne & Lawrence (1983)

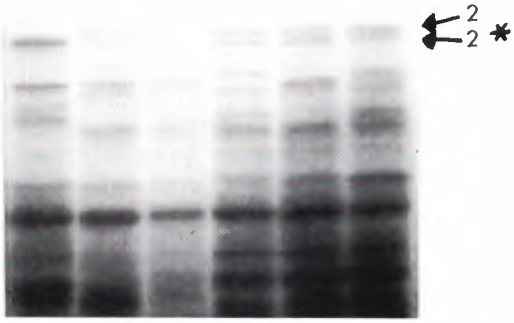

Fig. 3. 5% SDS-PAGE of 6 spring wheat breeding lines. HMW glutenin subunits 2 and 2* are marked on the figure

RESULTS

Typical patterns of 10% SDS-PAGE are shown in Fig. 2 for 10 of the breeding lines. The HMW subunits of glutenin can easily be identified as the four to five bands at the top of the gel. Classification numbers according to the nomenclature of Payne & Lawrence (1983) are given in the figure. Separations of 5% SDS-PAGE are shown in Fig. 3. Subunits 2 and 2* are separated and marked with arrows.

Allelic variation of HMW glutenin subunits in Norwegian spring wheat

The composition of HMW subunits of glutenin in Norwegian spring wheat varieties is given in Table 2. There is only limited allelic variation in the Glu-1 loci of these varieties. The variation includes subunits 2* and 1 coded from Glu-A1, subunits 7+8, 7+9 and 6+8 coded from

Table 2. The HMW glutenin subunit composition of Norwegian wheat varieties

Variety	Glu-A1	Glu-B1	Glu-D1
Børsum	2*	7+8/7+9	2+12
Ås	2*	7+9	2+12
Frøya	2*	7+8/7+9	2+12
Fram I	2*	7+8	2+12
Fram II	2*	7+8	2+12
Snøgg II	2*	7+8/7+9	2+12
Trym	2*	7+9	2+12
Norrøna	2*	7+8	5+10
Nora	2*	7+8	5+10
Rollo	2*	7+8	5+10
Møystad	2*	6+8	2+12/5+10
Runar *	2*	7+8	5+10
Reno *	1	7+9	5+10

* Currently grown commercially.

Glu-B1, and subunits 2+12 and 5+10 coded from the Glu-D1 locus. Four of the varieties, Børsum, Frøya, Snøgg II and Møystad, had double sets of subunits coded from the Glu-B1 or the Glu-D1 loci. This could be due to different biotypes that may exist in these varieties, or to impurities in the grain samples from other varieties.

The HMW glutenin subunit composition of the 252 breeding lines showed more extensive allelic variation compared with the varieties. The allelic variation comprises three alleles of the Glu-A1 locus, seven alleles of the Glu-B1 locus, and two alleles of the Glu-D1 locus. Breeding lines being impure or still segregating in HMW glutenin subunits were detected. When these lines were excluded, 24 different combinations of HMW subunits of glutenin were found. The number of lines of the various combinations of HMW glutenin subunits are given in Table 3. The HMW glutenin subunit combinations 2*, 7+8, 5+10, and 2*, 13+16, 5+10 were prevalent in this material, and were present in 40.5% of the breeding lines. The allelic frequencies at each gene locus are presented in Fig. 4. Only two lines contained the null-allele encoded by the gene locus Glu-A1;

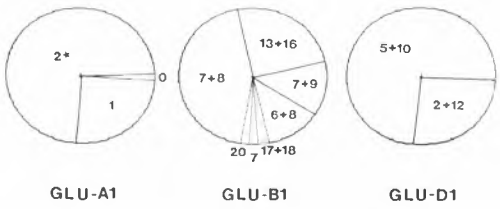

Fig. 4. Frequencies of the alleles at the three gene loci Glu-A1, Glu-B1, and Glu-D1. The data are from 212 pure and homzygot breeding lines

the rest had 2* or 1, of which 2* was the most dominant. Among the alleles encoded by Glu-B1, subunits 7+8 were most commonly followed by subunits 13+16. Subunits 20, 17+18 and 7 were rare in this material. Of the two alleles found at the Glu-D1 locus, subunits 5+10 were present in 76% of the lines, the rest had subunits 2+12.

Associations between HMW glutenin subunits and bread-making quality

The HMW glutenin subunit composition of breeding lines from the preliminary yield trials revealed that some subunits were present in very low frequencies. These were the Glu-1B encoded subunits 17+18, 20 and 7, and the null-allele coded from Glu-A1. The effects of these subunits on the bread-making quality could, therefore, not be tested properly in this material.

Analysis of variance

The breeding lines from the preliminary yield trials, which comprised a total of 177 lines, were grouped according to their HMW glutenin subunit composition, and analysed by an analysis of variance.

The breeding lines were first grouped according to the Glu-A1 alleles: subunit 1, subunit 2* and the null-allele. No significant differences were found between these groups, either in protein content or in the different quality tests (results not shown). The two lines that contained the null-allele were low in sedimentation

Table 3. The frequencies and numbers of the various combinations of HMW glutenin subunits found in the spring wheat breeding lines. The data are from 212 pure and homozygote breeding lines

	HMW subunits of glutenin			No. of lines	Frequencies %
	Glu-A1	Glu-B1	Glu-D1		
1	0	17+18	5+10	1	.5
2	0	7+8	5+10	1	.5
3	1	20	2+12	1	.5
4	1	6+8	2+12	2	.9
5	1	7+8	2+12	4	1.9
6	1	13+16	5+10	6	2.8
7	1	17+18	5+10	1	.5
8	1	6+8	5+10	2	.9
9	1	7	5+10	2	.9
10	1	7+9	5+10	13	6.1
11	1	7+8	5+10	18	8.5
12	2*	13+16	2+12	7	3.3
13	2*	17+18	2+12	2	.9
14	2*	6+8	2+12	6	2.8
15	2*	7	2+12	3	1.4
16	2*	7+9	2+12	2	.9
17	2*	7+8	2+12	22	10.4
18	2*	13+16	5+10	38	17.9
19	2*	17+18	5+10	4	1.9
20	2*	20	5+10	2	.9
21	2*	6+8	5+10	15	7.1
22	2*	7	5+10	3	1.4
23	2*	7+9	5+10	9	4.2
24	2*	7+8	5+10	48	22.6

volume. As the null-allele is reported to cause poor bread-making quality (Payne et al. 1984), these two lines were excluded from further analysis in order to prevent their haveing an influence on analyses of other subunits.

The lines were then grouped according to the subunits encoded by the Glu-D1 locus, 5+10 and 2+12. The averages of the different quality tests of these two groups are presented in Table 4. Lines containing subunits 5+10 had much higher sedimentation volumes than lines containing subunit 2+12. The lines with subunits 5+10 were higher in protein content, and this may have affected these results. However, the lines containing subunits 5+10 were also higher in specific sedimentation volume, strongly indicating differences in protein quality between these subunits. No differences in gluten content between the two groups were found, even though there were differences in protein content. When the preliminary yield trials from each year were analysed separately, similar results were obtained, but in 1986 only differences in Zeleny sedimentation volume were significant (results not shown).

The results from the Brabender's Extensigraph, carried out for the preliminary yield trial in 1985, were analysed in the same way as the Zeleny sedimentation volume. The breeding lines containing the HMW glutenin subunits 5+10 produced extensograms with higher resistance and with greater extensibility than the lines containing subunits 2+12 (Table 4b). No differences in protein content were found between these two groups in this subset of the data.

The differences found in the sedimentation volumes and in the extensograms between lines containing subunits 5+10 and 2+12 were not reflected

in the baking tests available for the preliminary yield trial in 1984 (Table 4c).

The lines were further subdivided according to the Glu-B1 alleles within the groups of subunits 5+10 and 2+12 as shown in Table 4 (d, e, f). For the lines containing subunits 5+10, significant differences in Zeleny sedimentation volume were found between the groups of the Glu-B1 alleles. When specific Zeleny

Table 4. Differences in technological properties between the lines from the preliminary yield trials grouped according to their alleles at the Glu-D1 (a, b, c) and the Glu-B1 loci (d, e, f). a) and d) are results from 1984, 1985 and 1986; b) and e) are results from 1985; c) and f) are results from 1984

	Glu-D1 encoded HMW glutenin subunits 5+10	2+12	
a) P	13.2	12.7	**
Z	50	37	***
SZ	44	36	***
G	30.7	30.1	NS
No. of lines	122	42	
b) E_{res}	326	211	***
E_{ext}	188	169	**
E_{area}	94	60	***
P	13.1	13.3	NS
No. of lines	50	8	
c) LV	830	826	NS
P	12.7	12.1	*
No. of lines	33	24	

	Lines with subunits 5+10 grouped according to the Glu-B1 encoding subunits: 7+8	7+9	6+8	13+16		Lines with subunits 2+12 grouped according to the Glu-B1 encoding subunits: 7+8	7+9	6+8	13+16	
d) P	13.0	13.1	13.3	13.5	NS	12.9	13.8	11.5	12.8	*
Z	45	49	51	55	**	40	52	28	39	NS
SZ	42	44	45	47	**	37	40	34	37	NS
G	29.8	30.3	30.7	31.3	NS	29.6	40.9	28.3	30.4	**
No. of lines	34	13	15	38		16	1	7	4	
e) E_{res}	284	294	351	351	**					
E_{ext}	180	178	182	199	*					
E_{area}	79	82	95	105	**					
P	12.8	13.1	13.2	13.3	NS					
No. of lines	14	5	6	22						
f) LV	834	764	799	865	NS					
P	12.9	12.4	12.6	12.9	NS					
No. of lines	13	4	2	6						

* $0.01 < P < 0.05$ ** $0.001 < P < 0.01$ *** $P < 0.001$

P = Protein content (%), Z = Zeleny sedimentation volume (ml), SZ = Specific Zeleny sedimentation volume (ml), G = Gluten content (%), E_{res} = Extensogram resistance (BE), E_{ext} = Extensogram extensibility (mm), E_a = Extensogram area (CM^2), LV = Loaf Volume (ml).

sedimentation volume was analysed, the following ranking of the Glu-B1 subunits was obtained: 13+16 > 6+8 = 7+9 > 7+8. No significant differences in gluten content were found between these groups.

In the preliminary yield trial in 1985, the lines with the HMW glutenin subunits 13+16 or 6+8 produced extensograms of highest resistance, and the lines with subunits 13+16 produced extensograms of greatest extensibility (Table 4e). There were no differences in protein content between these groups in this subset of the data. Results from baking tests, available for lines grown in 1984, revealed no differences in loaf volume between the Glu-1B alleles (Table 4f).

The 42 lines from the preliminary yield trials containing subunits 2+12 were also subdivided according to the Glu-B1 alleles and analysed for protein content, sedimentation volume and gluten content (Table 4d). There were no differences in sedimentation volume between the groups. However, significant differences in protein content were found, and the one line with subunits 7+9 was especially high in both protein content and gluten content.

Multiple regression analysis

The data from the preliminary yield trials were analysed by stepwise multiple regression analysis using the HMW subunits of glutenin and the protein content as x-variables and the Zeleny sedimentation volume as the dependent y-variable. Only subunits 5+10, 2+12 and 13+16 contributed significantly to the determination of Zeleny sedimentation volume in addition to the protein content. Subunits 5+10 and 13+16 had positive regression coefficients, whereas subunits 2+12 had negative regression coefficients. The overall R^2 was .32 when only HMW subunits of glutenin were taken into consideration, increasing to .65 when the protein content was added. When the «year of cultivation» was added as another x-variable, the R^2 increased further to .74.

When the specific Zeleny sedimentation volume was analysed as the dependent y-variable using the HMW glutenin subunits as x-variables, subunits 5+10, 13+16 and 2+12 contributed significantly to the determination of y. The regression coefficients of these alleles were similar to those obtained for the analysis of Zeleny sedimentation volume. The overall R^2 was 0.27.

Partial Least Squares (PLS) regression analysis

The data from the preliminary yield trials were analysed by PLS regression analyses using the HMW subunits of glutenin and the protein content as x-variables and Zeleny sedimentation volume as the y-variable. The predicted variances in Zeleny sedimentation volume, evaluated by «cross-validation», are shown in Fig. 5 for data from each respective year (a, b, c) and for the pooled data from all three years (d). Only one «PLS-factor» is included in all analyses, as determined by the cross-validation test. The results

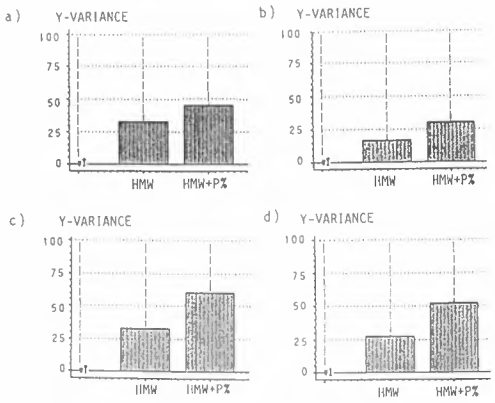

Fig. 5. Predicted variances in Zeleny sedimentation volume for analyses of the preliminary yield trials in 1984 (a), 1985 (b), 1986 (c) and for pooled data over years (d). The variables of the HMW subunits of glutenin alone (HMW) and in addition to the protein content (HMW+P%) are used as x-variables in the analyses

revealed that the proportion of variance in Zeleny sedimentation volume predicted by the PLS regression varied between the three years. The contributions from the HMW subunits of glutenin were 34%, 17% and 33% for the years 1984, 1985 and 1986, respectively. For the pooled data, 27% of the variation in Zeleny sedimentation volume was predicted by the HMW subunits of glutenin, and this proportion increased to 52% when the protein content was included as another x-variable.

The «loadings» of the x-variables for the analyses of Zeleny sedimentation volume as a function of both the HMW glutenin subunits and the protein content are shown in Fig. 6. High positive loadings were found for the protein content and the HMW glutenin subunits 5+10 and 13+16, revealing that a high protein content and the presence of these subunits are the important factors in producing high sedimentation volumes in the material analysed. Subunits 2+12, the only allelic alternative to subunits 5+10 in this material, had the most negative loading.

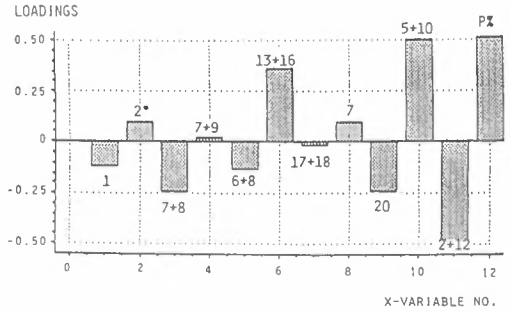

Fig. 6. The «loadings» of the x-variables for the first PLS-factor for the analyses of Zeleny sedimentation volume as the y-variable. HMW subunits of glutenin and the protein content are used as x-variables. The data are from preliminary yield trials in 1984, 1985 and 1986

For the pooled data from all three years, 25% of the variance in specific Zeleny sedimentation volume was predicted by the HMW glutenin subunit composition. No additional predictive information was obtained when the protein content was included as another x-variable. The loadings of the HMW glutenin subunits for this analysis were almost identical to those shown in Fig. 6.

DISCUSSION

The 14 Norwegian spring wheat varieties analysed for their HMW glutenin subunit composition had a very limited allelic variation in the Glu-1 loci. It may be assumed from these results that the land races, which are the original sources of germplasm for the older varieties, had little variation in the composition of HMW subunits of glutenin, and that this variation was probably limited to subunits 2*, 7+8, 7+9, and 2+12. Subunits 5+10 were first introduced into the Norwegian varieties Norrøna and Nora through the Finnish variety Sopu. According to Sontag et al. (1986), Sopu acquired these subunits through the Canadian bread-wheat variety Marquis.

The greater variation in Glu-1 alleles found in the breeding lines reflects the more extensive use of foreign germplasm in crosses during the later decades. In particular, germplasm from CIMMYT has been used in the Norwegian Spring Wheat Breeding Programme. It is likely that subunits 17+18 have been transferred from this material. Subunits 13+16 occur frequently in the analysed breeding lines. By examining the pedigrees of these breeding lines, it became evident that these subunits were also transferred to the Norwegian breeding material through a CIMMYT line Sonora/TZPP//Nainari. The prevalence of subunits 5+10 in the breeding lines probably reflects the extent to which the varieties Runar and Reno have contributed to the pedigrees of subsequent lines.

Associations to bread-making quality

The relationship between the composition of HMW subunits of glutenin and the bread-making quality, the latter measured by Zeleny sedimentation volume, extensogram, gluten content and loaf volume, has been examined in this study. Zeleny sedimentation volume and extensogram measure properties of the proteins important for the bread-making quality. Loaf volume, usually considered as the final test for bread-making quality, are in contrast influenced by many components of the wheat kernel as well as the proteins, and by the test-baking methods. All these quality tests are affected by variations in grain protein content as well as variations in protein quality. The calculations of specific Zeleny sedimentation volumes, which successfully removed the effects of the variation in protein content in this material, may be a possible way to obtain a parameter which measures the protein quality of wheat samples only.

Several quality-related HMW subunits of glutenin could be identified when Zeleny sedimentation volume and extensogram were used as the quality tests. The results of these tests showed that lines with subunits 5+10 were better in bread-making quality than those with subunits 2+12. This was evidenced in all the statistical analyses carried out, and concurs with the results of Burnof & Bouriquet (1980), Payne et al.(1981a), Moonen et al. (1982), Payne et al. (1984), Branlard & Dardevet (1985), Payne et al. (1987a), Campbell et al. (1987), Cressey et al. (1987) and Ng & Bushuk (1988). Of the HMW subunits encoded by the 1B chromosome present in this material, subunits 13+16 were most strongly related to good bread-making quality. These subunits were found in very low frequency in a world collection of 300 wheat varieties (Payne & Lawrence 1983), indicating that they are rare in most breeding materials. In an earlier work, these subunits were found to provide better bread-making quality than subunits 6+8 (Payne et al. 1981a). Of the other Glu-1B encoded HMW subunits of glutenin present in this material, both subunits 6+8 and subunits 7+9 seem to provide better protein quality than subunits 7+8. However, these results were only significant when lines with subunits 5+10 were analysed (Table 4 d, e), and they are in disagreement with those of Payne et al. (1984), who reported subunits 7+8 as providing better protein quality than subunits 7+9 and 6+8. When combined with the HMW glutenin subunits 2+12, however, subunits 6+8 seemed to provide lower sedimentation volume than subunits 7+8, but this difference was not significant. As the number of lines containing subunits 6+8 and 7+9 is low in this material, further examination of these subunits needs to be carried out.

The differences in quality found in Zeleny sedimentation volume and extensogram were reflected neither in the gluten content nor in the baking tests. As pointed out by Ringlund et al. (1986), the gluten content and the Zeleny sedimentation volume reflect different protein interactions of the gluten complex, both contributing different information to the bread-making quality. The Zeleny sedimentation volume is probably mainly influenced by the glutenin, as has been reported for the SDS-sedimentation volume (Payne et al. 1987b). In contrast, the gluten content is apparently a measure of the quantity of the gliadin and the glutenin as well as other flour components involved in the gluten complex. As for the baking tests, the differences found in protein quality attributable to the HMW glutenin subunit composition may have been masked by the influence of other components in the flour. Furthermore, as standardized test-baking methods are used, it is also possible that lines with good protein quality do not reach their potential under the baking conditions used and, therefore, underperform on baking.

The results from the stepwise multiple regression analysis revealed that the HMW glutenin subunit composition accounted for 32% of the variance in Zeleny sedimentation volume. However, since some HMW glutenin subunits and the protein content were intercorrelated in this material, this value of explained variance might have been overestimated. From the PLS regression analysis, which is designed to deal with intercorrelations in the data, 27% of the variance in sedimentation volume was predicted by the subunit composition for new samples. Approximately similar estimates were also found when specific Zeleny sedimentation volume was analysed (27% by the multiple regression analysis and 25% by the PLS regression analysis). It is therefore concluded that the HMW glutenin subunits account for about 25-27% of the variance in Zeleny sedimentation volume in this material. These estimates are much lower than those reported for UK - and Spanish-grown wheat varieties (Payne et al. 1987a; Payne et al. 1988). One reason for this could be that there is less variation in the Glu-1 alleles present in the Norwegian breeding lines as compared with varieties from the UK and Spain. In particular, some of the Glu-1 alleles which are related to poor protein quality, such as the null-allele, subunits 4+12, 3+12 and 7 (Payne et al. 1984), are missing or occur in very low frequencies in the Norwegian breeding lines.

In this study, collections of advanced breeding lines not selected for bread-making quality have been used in order to find associations between individual HMW subunits of glutenin and bread-making quality. However, in another wheat material, consisting of the breeding lines from the main yield trials, no such associations were found (Uhlen et al. 1987). This may be an effect of the selection for quality which these lines are subjected to, giving a narrower variation in bread-making quality. It is possible that lines containing «poor» HMW glutenin subunits but «good» subunits of the other storage protein groups have been accepted in this selection. Preferably, examination of the relationship between storage protein subunits and the bread-making quality should be carried out on wheat material not selected for bread-making quality. Furthermore, care should be taken when analysing collections of breeding lines because of possible irrelevant correlations attributable to pedigree associations. To minimize this risk, a large number of breeding lines from a number of crosses should be analysed. One advantage of using such collections is the broader spectrum of genetic background in which the individual polypeptides are tested, which provides results of a more general validity.

According to previous studies (Payne et al. 1984, 1987a), the prevalent HMW subunits of glutenin found in the Norwegian breeding lines are «good-quality» subunits, and should generally give a flour of high elasticity. In the future quality wheat breeding in Norway, care should be taken to keep these good combinations of HMW subunits of glutenin in the breeding materials. If parental lines with subunits related to poor quality are used, it would be advantageous to select against these subunits in early generations by using SDS-PAGE as a screening test.

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QUANTITATIVE ANALYSIS OF HIGH MOLECULAR WEIGHT GLUTENIN SUBUNITS PRESENT IN NORWEGIAN WHEATS

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The electrophoretic patterns of HMW glutenin subunits for 111 spring wheat breeding lines were analysed quantitatively by densitometer scanning. Significant differences in quantity between the various subunit bands were found. The following ranking of the subunit groups according to subunit peak areas was obtained: 1B x > 1D y = 1D x > 1A x = 1B y. One exception from this ranking was found to be the 1B x-subunit 6, which had about half the band intensity of the other 1B x- subunits. The total peak area of the HMW glutenin subunits was positively correlated with grain protein content. Additional variation in quantity of the HMW subunits of glutenin was found to have a slight effect on the variation in Zeleny sedimentation volume. It is concluded that selection for bread-making quality according to the electrophoretic patterns of HMW glutenin subunits can be made qualitatively without taking the quantity of the subunits into consideration.

Key words: Bread-making quality, densitometry scanning, glutenins, SDS-PAGE, *Triticum aestivum*, wheat.

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The high molecular weight (HMW) subunits of glutenin comprise the higher molecular weight polypeptides of the glutenin aggregates. The glutenin aggregates are part of the wheat storage proteins. The genes encoding the HMW subunits of glutenin are positioned in three complex loci (Glu-A1, Glu-B1 and Glu-D1) close to the centromere on the long arm of the homeologous chromosomes 1A, 1B and 1D (Bietz et al. 1975; Lawrence & Shepherd 1980; Payne et al. 1980; Lawrence & Shepherd 1981). The allelic variation found in these loci includes four alleles at the Glu-A1 locus encoding either a single subunit or no subunit, 12 alleles at the Glu-B1 locus encoding either one or two subunits, and six alleles at the Glu-D1 locus encoding two subunits (Payne & Lawrence 1983; Sontag et al. 1986; Lawrence 1986). Thus, each variety contains from three to five HMW subunits of glutenin. The allelic variation found in these three loci have been classified by Payne & Lawrence (1983).

Two genes are found to be present in each of the loci encoding HMW glutenin subunits (Harberd et al. 1986). The subunits encoded from these two genes are classified as «x»- and «y»-type subunits (Payne et al. 1981b), the x-type subunits being those of slower mobility on the

SDS-polyacrylamide gel. Varieties of hexaploid wheat have one 1B x-, one 1D x- and one 1D y-subunit, whereas 1B y- and 1A x-subunits are expressed for some alleles and silent for others. The 1A y-subunits appear to be silent in hexaploid wheat.

The HMW subunits of glutenin constitute only a small proportion of the storage proteins (Payne et al. 1984), but they exert a pronounced effect on the glutenin elasticity and the bread-making quality of the wheat flour (Payne et al. 1987a, 1988). A number of studies have shown that different HMW glutenin subunit alleles make different contributions to the bread-making properties (Payne et al. 1979; Burnouf & Bouriquet 1980; Payne et al. 1981a; Moonen et al. 1982; Payne et al. 1984; Branlard & Dardevet 1985; Campbell et al. 1987; Cressey et al. 1987; Ng & Bushuk 1988; Uhlen 1990). Small differences in the subunit amino acid sequences, which give important differences in the secondary structures of the HMW glutenin subunits, are suggested as an explanation for the variation in quality. Recently published nucleotide sequencing studies of the Glu-D1 encoded subunits 5+10 and 2+12 support this theory. The better quality of subunits 5+10 was associated with a more regular β-turn conformation of subunit 10, providing better elastic properties (Goldsbrough et al. 1988), and also with an extra cysteine residue of subunit 5 compared to subunit 2, affecting the crosslink patterns of the gluten structure (Greene et al. 1988).

Studies have also revealed that lack of expression of one or several HMW glutenin subunits in wheat lines can cause dramatic loss of bread-making quality of the flour (Payne et al. 1987b; Lawrence et al. 1988). In the study by Lawrence et al. (1988), significant relationships were found between the total quantity of the HMW glutenin subunits, measured by densitometer scanning, and bread-making quality. Furthermore, the introduction of an extra HMW glutenin subunit into hexaploid wheat, the 1A y-subunit from *Triticum thaoudar*, is found to produce increased gluten strength and reduced dough stickiness (Rogers et al. 1989). The results from these studies indicate that the total amount of HMW glutenin subunits in the flours may be of importance for the quality.

In a study examining the quantitative variation of specific HMW glutenin subunits by densitometer scanning, Payne et al. (1981b) found an approximately constant relationship between the subunit peak areas of several subunits. Except for 1B x- subunit 6, which unlike the other 1B x-subunits, was found to bind much less dye, the relative intensities of the subunit bands could be summarized as 1B x > 1D x = 1D y > 1A x (when present) > 1B y. Galili & Feldman (1983) also found similar relationships between band intensities of the HMW glutenin subunits belonging to the various subgroups. From their results the following relative intensities of the subunit bands could be deduced: 1D x = 1D y > 1A x = 1By. The intensity of the 1B x-subunits varied from twice as much to one-half of the band intensity of the 1D x- and 1D y-subunits. Part of this variation was due to the significantly lower intensity of subunit 6. Galili & Feldman (1983) also found the total quantities of the HMW glutenin subunits to be positively correlated to the grain protein content of the wheat samples.

This study was set up to examine the quantitative variation of the HMW glutenin subunits present in Norwegian wheat materials. An attempt is made to relate the total amounts of HMW glutenin subunits to the bread-making quality.

MATERIALS AND METHODS

The plant materials

The plant material analysed comprised 138 advanced spring wheat breeding lines (F_7-F_{10}) grown at the Department

of Crop Science, Agricultural University of Norway, Ås, in 1985 and 1986. Sixty-four of the lines were grown in 1985, the rest (74 lines) were grown in 1986. These lines were part of the material analysed by Uhlen (1990) to investigate the composition of HMW glutenin subunits in Norwegian wheat materials.

Fractionation and quantification of HMW subunits of glutenin

Total protein extracts made from wholemeal flour from Falling Number's Laboratory mill 3100 were fractionated by 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE). The method of Payne et al. (1980) with modifications as described by Uhlen (1990) was used. The HMW glutenin subunits were classified according to the numbering system of Payne & Lawrence (1983). The subunits 2 and 2* are usually not separated by 10% SDS-PAGE. Additional 5% SDS-PAGE as described by Payne et al. (1987a) were carried out for the breeding lines that possibly contained these two subunits.

The electrophoretic patterns of the HMW glutenin subunits were scanned on a Shimadzu CS 930 scanning densitometer at wavelength 570 nm, and the peak area of each subunit was automatically calculated. As the HMW glutenin subunits 17 and 18 are poorly separated by the 10% SDS-PAGE used, the peak area of both subunits was calculated by the densitometer scanning. The total peak area of HMW glutenin subunits was calculated by adding the subunit peak areas of each breeding line.

Evaluation of protein content and quality

Protein content was determined by NIR (Ringlund 1978) and presented on a dry weight basis. The Zeleny sedimentation test, performed by the method of AACC (1983) on white flour obtained from a Brabender Quadromat Junior mill, was used for evaluation of bread-making quality.

Statistical analyses

Data were analysed statistically using the software package MSTAT (Nissen & Mosleth 1986). Averages, standard deviations, 95% confidence intervals and simple correlation coefficients were calculated. Data were also analysed by stepwise multiple regression analysis.

RESULTS

Electrophoretic patterns of the HMW subunits of glutenin for six breeding lines are shown in Fig. 1. Classification

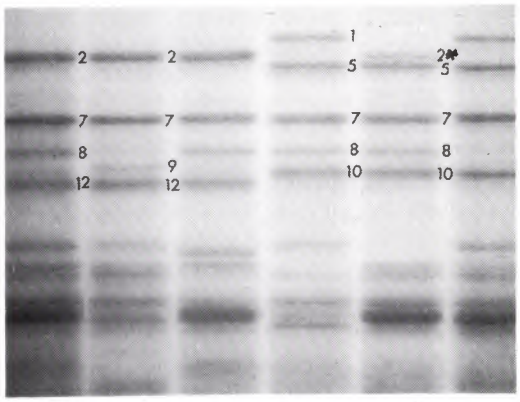

Fig. 1. Electrophoretic patterns of the HMW glutenin subunits for six of the breeding lines. Classification numbers are according to Payne & Lawrence (1983). The x- and y-subunits of the various Glu-1 loci are given

numbers according to the nomenclature of Payne & Lawrence (1983) are given in the figure. Of the 138 breeding lines analysed for their HMW glutenin subunit composition, some had double sets of HMW glutenin subunits encoded from the same locus. This may be due to mechanical mixtures of the grain samples, or to some heterozygosity in the lines. Only the breeding lines with single sets of subunits encoded from each loci, totally 111 lines, were used for the quantitative analysis of HMW glutenin subunits. The HMW glutenin subunit com-

position of these 111 lines is listed in Table 1. Fig. 2 shows the densitometer scans for four of these breeding lines.

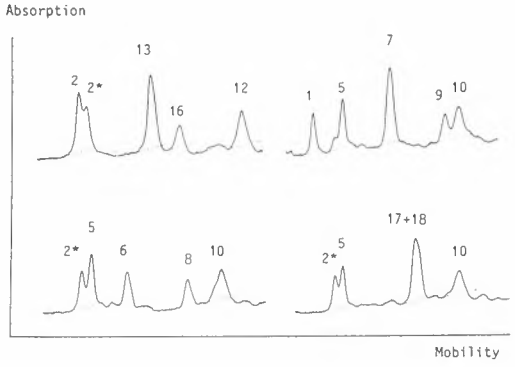

Fig. 2. Densitometer scans of HMW glutenin subunits for four of the breeding lines

The average peak areas of the various HMW subunits of glutenin, shown in Fig. 3, reveal significant differences between the subunits. Subunits 7, 13 and 17+18, encoded by Glu-B1, had the highest peak areas, significantly higher than any of the other subunits. The Glu-A1 encoded subunits 1 and 2*, and the Glu-B1 encoded y-type subunits 16, 8 and 9 were found to have the smallest peak areas. The peak areas of these subunits were all significantly smaller than subunits 10, 12, 2 and 5, encoded by Glu-D1. The average peak area of subunit 6, an x-type subunit coded from the Glu-B1 locus, was similar in size to the 1D x- and the 1A x-subunits.

Little variation was found in average protein content between the breeding lines containing the various HMW glutenin subunits. The lines with subunits 13+16 were highest in protein content (13.9%), whereas the lines with subunits 17+18 were lowest (13.1%). The average protein contents of breeding lines containing other subunits varied from 13.2% to 13.6%. Thus, the variation in protein content of the breeding lines is not likely to have affected the variation in peak areas of the various HMW glutenin subunits in the material analysed.

For HMW glutenin subunit 8, the

Table 1. The different compositions of HMW glutenin subunits occurring in the material analysed, and the number of breeding lines of the various composition. Data are from the 111 pure and homozygote breeding lines

	HMW subunits of glutenin			No. of lines
	Glu-A1	Glu-B1	Glu-D1	
1	0	17+18	5+10	1
2	2*	17+18	5+10	2
3	2*	7	2+12	2
4	2*	7	5+10	3
5	2*	7+8	2+12	9
6	2*	7+8	5+10	20
7	2*	13+16	2+12	3
8	2*	13+16	5+10	29
9	2*	6+8	2+12	2
10	2*	6+8	5+10	10
11	2*	7+9	2+12	1
12	2*	7+9	5+10	4
13	1	7	5+10	2
14	1	7+8	5+10	8
15	1	13+16	5+10	6
16	1	6+8	2+12	1
17	1	6+8	5+10	2
18	1	7+9	5+10	6

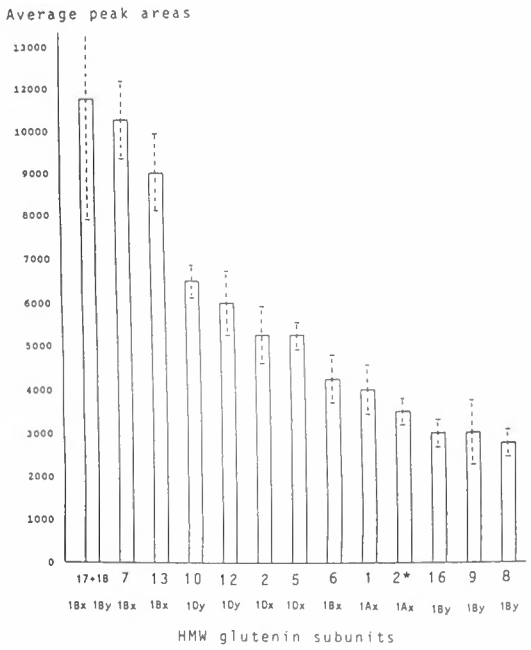

Fig. 3. Average peak areas of the various HMW glutenin subunits. 95% confidence intervals are indicated for each subunit

average peak area was calculated when this subunit was linked to either subunit 7 or subunit 6. The results revealed that the peak area of subunit 8 of the 6+8 allele was significantly higher than that of subunit 8 of the 7+8 allele (Table 2). For the HMW glutenin subunit 7 of the alleles 7+8, 7+9 and 7, no significant differences in band intensity were found.

Significant correlations were found between the total subunit peak areas for each breeding line on the one hand and the protein content ($r=0.25$, $P < 0.01$) and the Zeleny sedimentation volume ($r=0.28$, $P < 0.01$) on the other. The data were also analysed by stepwise multiple regression analysis using the protein content and the total subunit peak areas as the x-variables and Zeleny sedimentation volume as the y-variable. A small, but significant contribution to the determination of Zeleny sedimentation volume was then found from the total subunit peak areas ($R^2=0.03$ $P < 0.01$) in addition to the protein content.

DISCUSSION

The quantitative variation of HMW glutenin subunits has been examined in this study using dye intensities of the subunit bands digitalized by densitometer scanning as a measure of quantity. The binding capacity of the dye (Coomassie Brilliant Blue) is known to depend on the amino acid composition of the polypeptides. As the amino acid composition of various HMW glutenin subunits is almost identical (Shewry et al. 1984; Moonen et al. 1985), the differences in band intensity found between HMW glutenin subunits are most likely caused by differences in quantity.

The results revealed relatively constant relationships between the subunit band intensities of various HMW glutenin subunits belonging to the different subgroups. The following ranking of the subunit groups according to subunit peak areas was obtained: 1B x > 1D y = 1D x > 1A x = 1B y. One exception was the 1B x-subunit 6, which had only about half the band intensity of the other 1B x-

Table 2. Average peak areas of the HMW glutenin subunit 8 when combined with subunit 6 and 7. 95% confidence intervals are given in parentheses

	HMW glutenin subunit 8	
	of the 6+8 allele	of the 7+8 allele
Average peak area	4574 (± 1340)	2519 (± 483)
No. of lines	10	19

subunits. The results are in accordance with the results of Payne et al. (1981b) and Galili & Feldman (1983).

Only one structural gene appears to be present per HMW glutenin subunit (Harberd et al. 1986). Thus, the observed differences in quantity cannot be explained by variation in the number of structural genes of the specific subunits. As has been pointed out by Galili & Feldman (1985), the constancy in the relative proportions of HMW glutenin subunits under different genetic backgrounds indicates that the rate of expression is an inherited property of the structural genes themselves.

The higher quantity of subunit 8 when combined with subunit 6 than when combined with subunit 7 was also observed in the study by Payne et al. (1981b). Furthermore, Holt et al. (1981) found differences in the isoelectric point of subunit 8 when linked to its different counterparts. This may indicate that what has hitherto been regarded as one subunit (subunit 8) could actually be two structurally different subunits of almost identical molecular weight.

The peak areas of the HMW glutenin subunits were positively correlated to the protein content. As the Zeleny sedimentation volume is also highly affected by the protein content, the positive correlation found between the subunit peak areas and Zeleny sedimentation volume was expected. The multiple regression analysis revealed that the total peak areas of HMW glutenin subunits had a small, but significant effect on the Zeleny sedimentation volume in addition to the protein content. Considerably greater effects on Zeleny sedimentation volume, however, were found for the qualitative variation in HMW glutenin subunit composition in this material (Uhlen 1990). These results suggest that differences in the biochemical properties of the subunits seem to be more important than the overall quantity of HMW glutenin subunits. As the relative quantities of the different HMW glutenin subunits were found to be approximately constant from one genotype to another, information on the presence or absence of the various HMW glutenin subunit alleles along with the protein content may contain the main information on the total quantity of HMW glutenin subunits in genotypes. Hence, selection for bread-making quality according to the electrophoretic patterns of HMW glutenin subunits in the breeding programmes should be carried out qualitatively, on the basis of the presence or absence of the specific subunits.

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IDENTIFICATION OF QUALITY-RELATED GLIADINS AND PREDICTION OF BREAD-MAKING QUALITY OF WHEAT FROM THE ELECTROPHORETIC PATTERNS OF GLIADINS AND HIGH MOLECULAR WEIGHT SUBUNITS OF GLUTENIN

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Mosleth, E. & A.K. Uhlen 1990. Identification of quality-related gliadins and prediction of bread-making quality of wheat from the electrophoretic patterns of gliadins and high molecular weight subunits of glutenin. Norwegian Journal of Agricultural Sciences 4: 27-45. ISSN 0801-5341.

The present study reports investigation on the influence of gliadins, HMW glutenin subunits and protein content on Zeleny sedimentation volume and extensogram resistance and extensibility. The gliadins were separated by APAGE, the HMW glutenin subunits by SDS-PAGE, and the multivariate technique Partial Least Square (PLS) Regression were used as data-analytical tool. Six different gliadin bands were found to be strongly associated with bread-making quality. Three ω - gliadin bands and one γ-gliadin band, which correspond to the gliadin block Gld1B8, were positively associated with the quality tests. Two other gliadin bands, one ω -gliadin band and one γ-gliadin band, encoded by chromosome 1B, were negatively associated with the quality tests. It is concluded that the six gliadin bands found to be associated with bread-making quality may be useful as quality criteria in wheat breeding programmes in addition to the HMW glutenin subunits.

Key words: Bread-making quality, densitometry scanning, electrophoresis, gliadins, glutenins, multivariate analyses, *Triticum aestivum*, wheat.

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Abbreviations used in this paper:

APAGE	acid polyacrylamide gel electrophoresis
DNA	deoxyribonucleic acid
Ex 90 E	extensogram extensibility after 90 min
Ex 90 R	extensogram resistance after 90 min
Gli-1	complex loci on chromosomes 1 encoding gliadins and low molecular weight (LMW) glutenin subunits
Gli-2	complex loci on chromosomes 6 encoding gliadins
GLI (0-1)	qualitative variables expressing gliadin bands
GLI-SC	adjusted densitometer scan of APAGE pattern of gliadins
Glu-1	complex loci on chromosomes 1 encoding HMW subunits of glutenin
HMW	high molecular weight

HMW GLU (0-1)	qualitative variables expressing HMW glutenin subunits
HMW GLU (A)	scanning area of HMW glutenin subunits
LMW	low molecular weight
NIR	near infrared reflectance
PLS	Partial Least Squares
P%	protein content
RP-HPLC	reverse-phased high performance liquid chromatography
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SZEL	specific Zeleny sedimentation
ZEL	Zeleny sedimentation

The unique properties of bread doughs made from hexaploid wheat flour are primarily the result of the content and quality of the storage proteins in the flour. Storage proteins make up about 85% of the endosperm proteins in wheat kernels. When wheat flour is mixed with water, the storage proteins hydrate and form a gluten matrix, a complex, coherent network in which starch and other components are embedded. This gluten matrix is responsible for gas retention, which makes production of leavened bread possible. Improved quality of wheat for bread-making may be achieved by increasing the protein content or by improving the protein quality, or both (Finney & Barmore 1948). The protein content is strongly influenced by the growth conditions, whereas the protein quality is determined by the composition of the storage proteins which is mainly dependent on the genotype. Hence, concerning improvement in the bread-making quality of wheat by plant breeding, attention has been focused mainly on improvement in the storage protein composition.

The storage proteins consist of two main protein fractions; gliadin and glutenin. Classification of the storage proteins into these two groups is preferably carried out in accordance with the state of aggregation in dissociating media of these proteins. With this classification, gliadin occurs as a complex mixture of single polypeptides, whereas glutenin consists of polypeptides or «subunits» that are linked together mainly by disulphide bonds. The glutenin subunits are further divided into two groups based on their molecular weights; high molecular weight (HMW) subunits of glutenin and low molecular weight (LMW) subunits of glutenin (Payne et al. 1981b). Normally, the gliadin fraction is divided into the ω-, γ-, β-, and α-gliadins based on their mobility in acid polyacrylamide gel (Woychik et al. 1961). However, amino acid sequencing of gliadins and DNA sequencing of their genes have revealed a high degree of sequence homology between the α- and β-gliadins (Bartels & Thompson 1983; Anderson et al. 1984; Rafalski et al. 1984), suggesting that these two gliadin types belong to a common protein group.

The genes encoding the different types of polypeptides constituting the storage proteins have been mapped on the wheat chromosomes. Nine major loci have been identified at the homoeologous chromosome groups 1 and 6 (Bietz et al. 1975; Rybalka & Sozinov 1979; Lawrence & Shepherd 1980; Payne et al. 1980; Lawrence & Shepherd 1981; Payne et al. 1982; Dvorak & Chen 1983; Jackson et al. 1985). Figure 1 summarizes the present knowledge on the chromosomal location of these loci. Three loci located on the long arm of chromosomes 1A, 1B and 1D (the Glu-1 loci) encode HMW subunits of glutenin. Three other loci on the short arm of the same chromosomes (the Gli-1 loci) encode LMW glutenin subunits, ω- and γ- gliadins, whereas three loci on the short arm of chromosomes 6A, 6B and 6D (the Gli-2 loci) encode β- and α-gliadins. In addition, two minor loci encoding storage protein subunits have been found on the short arm of chromosomes 1A and 1B (Sobko 1984; Jackson et al. 1985). The polypeptides encoded from these loci have been classified as LMW subunits of glutenin, as they were strongly bound to glutenin in gel filtration studies (Jackson et al. 1985). Recently published results of Payne et al. (1988b), however, have revealed that

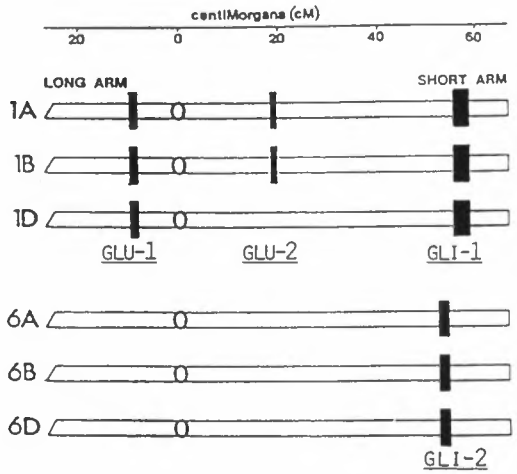

Figure 1. Chromosomal locations of genes encoding storage proteins in the wheat endosperm. The thickness of the vertical bars illustrates the relative complexity of these loci (Payne 1987)

these polypeptides are gliadin-type molecules, closely related to the ω-gliadins.

All the loci encoding storage protein polypeptides are composed of tightly linked genes which rarely recombine, i.e. they are complex loci. The number of genes encoding gliadins at Gli-1 and Gli-2 loci has been estimated to 9-15 and 9-12, respectively (Harberd et al. 1986). This is more than the number of major polypeptides expressed by these loci (Payne 1987). The explanation for this discrepancy may be the presence of pseudogenes, as reported for the Glu-1 loci (Payne et al. 1985). Another possibility is that some of the dominating polypeptides, in particular the γ-gliadins, may be products of two or more gene copies (Payne 1987).

As recombination is extremely rare between the genes encoding gliadins at the Gli-1 and the Gli-2 loci, the alleles at each of these loci will be inherited as linked groups or «blocks» (Sozinov et al. 1975; Baker & Bushuk 1978; Mecham et al. 1978; Sozinov & Poperelya 1980; Branlard 1983; Payne et al. 1984b). The gliadin polymorphism have been studied extensively by the use of one-dimensional starch gel electrophoresis by Sozinov & Poperelya (1980), who were able to catalogue a number of gliadin blocks encoded from the Gli-1 and the Gli-2 loci. This work has been extended further using polyacrylamide gel electrophoresis (Metakovsky et al. 1984, Metakovsky unpublished results). The most recently identified gliadin blocks of Metakovsky (unpublished results) are shown in Fig. 2.

For the HMW subunits of glutenin, approximately 25 different subunits have been identified in hexaploid wheat (Payne & Lawrence 1983; Lawrence 1986; Sontag et al. 1986). Each variety has three to five subunits (Lawrence & Shepherd 1980). Variation in the composition of HMW glutenin subunits among different genotypes has a pronounced influence on the bread-making quality (Payne et al. 1979, 1981a; Moonen et al. 1982; Payne et al. 1984a; Branlard & Dardevet 1985; Campbell et al. 1987; Cressey et al. 1987; Payne et al. 1987a, 1988a; Ng & Bushuk 1988; Uhlen 1990a). Today, the electrophoretic pattern of the HMW glutenin subunits is used for quality selections in wheat breeding programmes in several countries. As the one-dimensional electrophoretic technique used to fractionate these subunits is a fairly rapid method, and can be performed on the endosperm of a single wheat grain, selection based on the electrophoretic pattern can be carried out at an early stage in the breeding programme. The electrophoretic pattern can also be used as a guideline for selecting parents for new crosses.

The numbers of polypeptides belonging to gliadins and LMW subunits of glutenin are much larger than those belonging to HMW subunits of glutenin. Using two-dimensional electrophoresis, Wrigley & Shepherd (1973) found that each variety contains approximately 45 gliadins. For the LMW subunits of glutenin, each variety is reported to contain 14-16 subunits (Wall 1979). Investigations of the influence of gliadins and

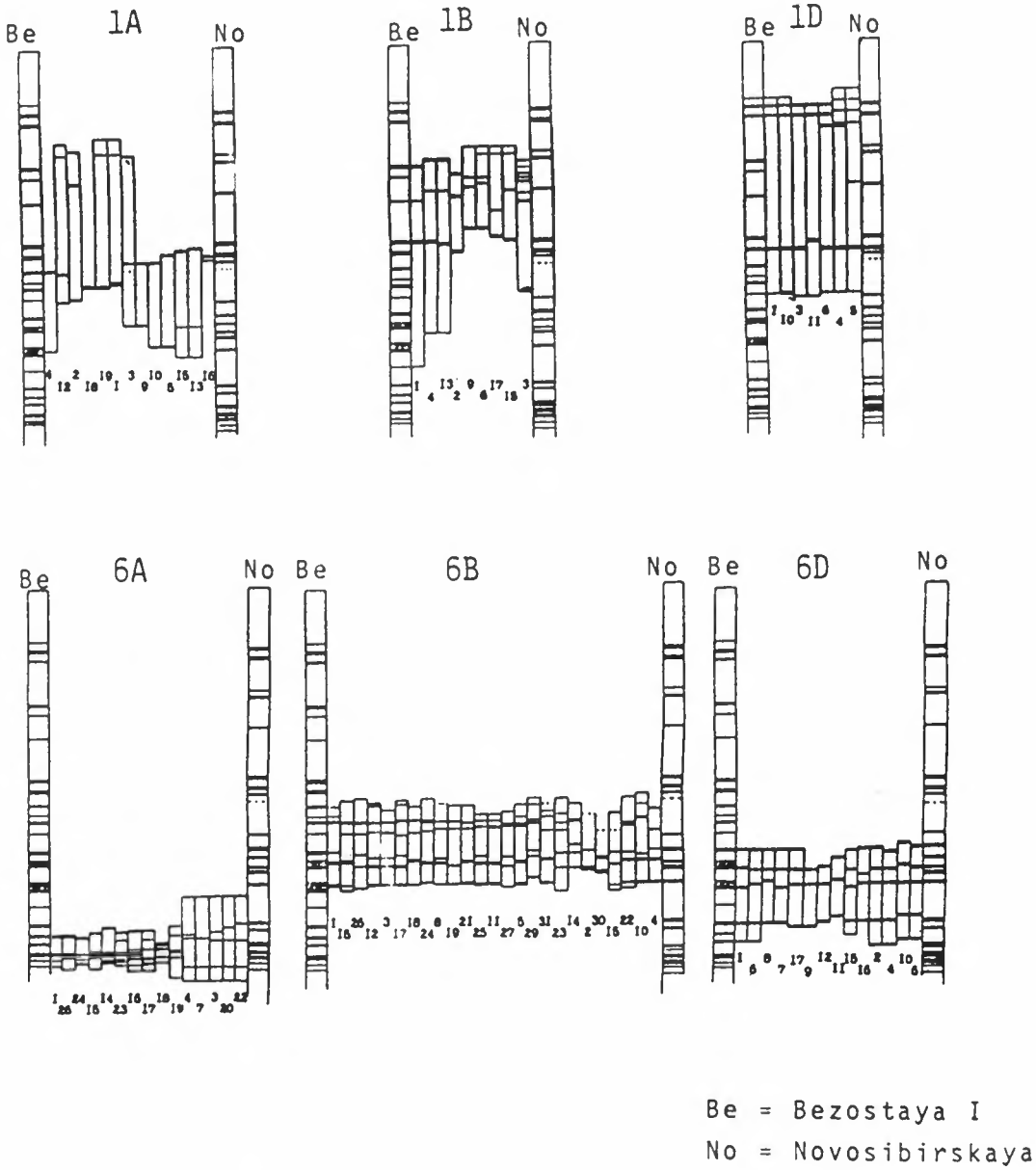

Figure 2. Gliadin blocks encoded from gene loci at chromosomes 1A, 1B, 1D, 6A, 6B and 6D identified on the basis of polyacrylamide gel electrophoresis (Metakovsky unpublished results)

LMW glutenin subunits on the bread-making quality are therefore more difficult than for the HMW glutenin subunits. Besides, attempts to summarize previous studies relating the gliadin compositions of varieties to the bread-making quality are impeded by the inconsistent nomenclature and the diffe-

rent electrophoretic techniques used. It should also be noted that positive or negative associations found between bread-making quality and ω- and γ-gliadins may be due to LMW subunits of glutenin (and vice versa), as the genes of these protein groups are closely linked. Another complicating factor is that one-dimensional gel electrophoresis does not resolve all the different gliadins adequately. This is particularly true of the α- and β-gliadins. A better resolution of the gliadins can be achieved by using two-dimensional techniques (Payne et al. 1984b).

On analysing a large number of progenies from many different crosses using one-dimensional electrophoresis, Sozinov & Poperelya (1980) were able to find consistent associations between several gliadin blocks and bread-making quality, the latter mainly determined by the Zeleny sedimentation test. However, as these gliadin blocks were identified using starch gel electrophoresis, they cannot be directly compared to gels of polyacrylamide (Metakovsky 1984), which are most commonly used today throughout the world.

Payne et al. (1987b) analysed F_5 progenies from a cross between two genotypes differing only in the 1A chromosome. They found significant differences in SDS-sedimentation volume corresponding to variation in the Gli-A1 locus, which encodes both gliadins and LMW subunits of glutenin. However, among the polypeptides coded for by the Gli-A1 locus, only LMW subunits of glutenin were found in abundance as gel protein in the SDS-sediment. It was therefore concluded that the differences found in the SDS-sedimentation volume between genotypes differing in the Gli-A1 locus primarily stemmed from the LMW subunits of glutenin. Other workers have found quality differences associated with gliadins encoded from the loci at chromosome 6 (Campbell et al. 1987). These loci encode gliadins only.

When analysing the effects of different gliadins or gliadin blocks on the quality parameters, problems arise because of variation in the genetic background in other storage protein alleles. In particular, the HMW subunits of glutenin may mask the effects of the gliadins. This problem can be overcome by using isogeneic lines differing only in one locus, or by analysing progenies from crosses of parental material differing in a few loci only. Another approach would be to analyse a large set of varieties with a wide range of quality types, and to include in the analyses variation in other storage proteins.

On investigating 177 Norwegian grown breeding lines for their composition of HMW subunits of glutenin, Uhlen (1990a) found that approximately 27% of the variance in Zeleny sedimentation volume could be accounted for by variation in the HMW glutenin subunit composition. In the present work the electrophoretic patterns of the gliadins as well as those of the HMW glutenin subunits of 97 of these breeding lines were studied. The relatively new multivariate technique, Partial Least Square (PLS) regression, was used to relate the electrophoretic patterns to bread-making quality. This method allows a large number of intercorrelated variables to be used simultaneously to describe the variation in quality parameters. Thus, all the information on the storage protein composition revealed by the two different one-dimensional gel electrophoretic techniques and the protein content could be taken into account in the data analyses.

The purpose of this paper was to identify possible quality-related gliadins or gliadin blocks, and to estimate their contribution to bread-making quality. A further aim was to generate calibration models for predicting bread-making quality for new varieties or breeding lines on the basis of the electrophoretic patterns of gliadins and HMW glutenin subunits.

MATERIALS AND METHODS

The plant material

The plant material analysed consisted of 97 advanced breeding lines (F_7-F_8) from the spring wheat breeding programme at the Department of Crop Science, Agricultural University of Norway. The breeding lines were grown in two different field trials, one in 1985 the other in 1986 consisting of 51 and 46 lines, respectively. The references used for these trials are «85B2» and «86B2». Both field trials were located at the experimental farm of the Department of Crop Science at Ås. No selection for bread-making quality has been applied to these breeding lines.

Evaluation of protein quality

Protein content (P%) was determined by NIR (Ringlund 1978), and is presented on a dry weight basis. The Zeleny sedimentation test (ZEL) was carried out according to AACC (1983) on white flour obtained from a Brabender Quadromat Junior mill. As ZEL is influenced by P% as well as by the storage protein composition, a quality parameter termed «specific Zeleny sedimentation volume» (SZEL) was calculated by adjusting ZEL for the linear relationship with P% as described by Uhlen (1990a). In order to study the rheological properties of the doughs, extensograms of the Brabender's extensograph were obtained for the lines in 85B2. These were taken at the Swedish Cereal Laboratory, Svalöf by their standard methods (ICC, nr. 115).

The protein composition

The compositions of HMW glutenin subunits and of gliadins were examined using gel electrophoretic techniques of protein extracts made of whole meal flour from a Falling Number's Laboratory mill 3100. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Payne et al. (1980) with minor modifications as described by Uhlen (1990a), was used for analysing the composition of HMW subunits of glutenin. The HMW glutenin subunits were classified according to the numbering system of Payne & Lawrence (1983). The gliadins were extracted in 70% ethanol and fractionated by one-dimensional polyacrylamide gel electrophoresis at pH 3.1 (APAGE) by the method of Bushuk & Zillman (1978). However, the concentration of hydrogen peroxide, used as a catalyst for the gel polymerization, was reduced according to Khan et al. (1985) to produce a firmer gel that gave a better resolution. Electrophoresis was carried out using the Pharmacia Gel Electrophoresis Apparatus GE-2/4 LS at 500 V. For 85B2, the running time for the electrophoresis was set to the time required for the marker to reach the bottom of the gel multiplied by a factor of 1.7. Improved resolution of the gliadin bands was obtained for 86B2 by increasing this factor to 1.8. The gels were stained as described for SDS-PAGE (Uhlen 1990a), and destained in 6% trichloracetic acid.

Two-dimensional electrophoresis (APAGE*SDS-PAGE) as described by Payne et al. (1984b) was carried out for a few breeding lines during a stay at PBI, Cambridge. Some of the gliadins are identified by the chromosome numbers (1A, 1B, 1D) of their genes according to Payne (pers. comm.).

Quantification of the electrophoretic patterns

The electrophoretic patterns of both the HMW subunits of glutenin and the gliadins were scanned on a Shimadzu scanning densitometer CS 930 at 570 nm. The peak area of each HMW glutenin subunit was calculated and expressed as one variable. For the gliadins, the whole scanning curve was quantified by manually tracing the scan curve with a digitizer HP 9111A Graphic Tablet. In order to avoid inaccuracy caused by variable electrophoretic conditions between different gels and between different tracks within each gel, three well-de-

fined gliadin bands were used as references and the longitudinal extensions of the digitized curves were adjusted according to these three bands. Adjustment for the background staining, which decreased gradually from the top of the gel to the bottom, was obtained by subtracting a linear function found for each electrophoretic track from the digitized scanning curve. The resulting curves were expressed as 156 variables. The gliadin bands of the one-dimensional APAGE were identified by the variable number corresponding to the highest position of the peaks on the adjusted scanning curves.

Data analyses

The multivariate data analytic technique, Partial Least Squares (PLS) regression (Wold et al. 1982; Martens 1985; Martens & Næs 1989), was used to investigate the relationship between the electrophoretic patterns of gliadins and HMW glutenin subunits and the bread-making quality tests. This method allows the electrophoretic patterns of the two protein groups to be used simultaneously to describe the variation in the quality tests.

The peak areas of the individual HMW glutenin subunits, the adjusted, digitized curve of the gliadins, and the protein content were used as x-variables and the bread-making quality tests as y-variables. The magnitude of the different x-variables varied considerably, and therefore all the variables were standardized to unit variance.

In the PLS regression analysis, information from all the x-variables is combined into a few basic variables or «PLS-factors» orthogonal to each other. The PLS factors are linear combinations of the original variables, estimated to give, in descending order, relevant predictions of the quality tests (the y-variables). Variable plots of the first PLS factors give information on how the different electrophoretic bands relate to the bread-making quality tests. Variables having strong positive «loadings» for the first PLS factor(s) are positively associated with quality, while those having strong negative loadings are negatively associated with quality.

The first PLS factors are used for estimating regression coefficients, whereas the last PLS factors are ignored as they contain noise and irrelevant prediction information. The regression coefficients estimated from the first PLS factors constitute a calibration model that can be used to predict the bread-making quality from the electrophoretic patterns and the protein content. When regression coefficients are estimated, prediction of the quality (the y-variable) is carried out as in multiple linear regression analyses.

The number of factors to be included in the calibration model is determined on the basis of a predictive cross-validation test (Wold 1978). With this method the samples are divided into a number of «Cross-Validation segments». One segment at a time is left out of the calibration and used for evaluation of the predictions. This is repeated so that all the breeding lines are left out once.

An overview of the different PLS regression analyses carried out in this study is given in Table 1. These analyses will be referred to according to the numbers given in the table. Zeleny sedimentation volume (ZEL) was used as the y-variable in the first three analyses. In analysis no. 1, protein content (P%) was used as the only x-variable. In analysis no. 2, both P% and the scanning areas of the different HMW glutenin subunits (HMW GLU (A)) were used as x-variables. This gives a total of 14 x-variables. In the third analysis the adjusted digitalized scanning curves of the gliadins (GLI-SC) were included as well, giving a total of 170 x- variables.

SZEL was used as the y-variable in data analyses nos. 4 and 5 (Table 1). In data analysis 4, HMW GLU (A) was used as the x-variables, and in analysis 5, the

x-variables were both HMW GLU (A) and GLI-SC.

PLS regression was also carried out on qualitative data of the electrophoretic patterns. The electrophoretic variables were transformed to 0-1 variables by being given the value «1» when a band was present and «0» when a band was absent. All the HMW glutenin subunits were included as 13 x-variables (HMW GLU (0-1)) in the qualitative data analysis no. 6, and SZEL was used as the y-variable. The gliadin bands found to be associated with bread-making quality in the previously described analyses were also transformed to qualitative variables. These variables were included in PLS analysis no. 7 as 6 x-variables (GLI (0-1)) in addition to the 13 HMW GLU (0-1) variables.

PLS regression analyses nos. 1 to 7 were carried out separately for 85B2 and 86B2. The two sets of data were also combined and analysed in equivalence with data analyses 3 and 5. The year of cultivation («YEAR«) was included as a second y-variable consisting of «0» for samples in dataset 85B2 and «1» for samples in dataset 86B2. These PLS regression analyses are given as nos. 8 and 9 in Table 1.

Table 1. An overview of the PLS regression analyses carried out in this study. X-variables are listed on the left, y-variables on the right. The variable numbers are given below each set of variables

	X-variables	Y-variables
Analysed for 85B2 and 86B2 separately:		
1	P%	ZEL
	1	1
2	HMW GLU (A) + P%	ZEL
	1-13 14	1
3	HMW GLU (A) + GLI-SC + P%	ZEL
	1-13 14-169 170	1
4	HMW GLU (A) S	ZEL
	1-13	1
5	HMW GLU (A) + GLI-SC	SZEL
	1-13 14-169	1
6	HMW GLU (0-1)	SZEL
	1-13	1
7	HMW GLU (0-1) + GLI (0-1)	SZEL
	1-13 14-19	1
Analysed for 85B2 and 86B2 combined:		
8	HMW GLU (A) + GLI-SC + P%	ZEL + YEAR
	1-13 14-169 170	1 2
9	HMW GLU (A) + GLI-SC	SZEL + YEAR
	1-13 14-169	1 2
Analysed for 85B2 only:		
10	HMW GLU (A) + GLI-SC + P%	Ex 90 R
	1-13 14-169 170	1
11	HMW GLU (A) + GLI-SC + P%	Ex 90 E
	1-13 14-169 170	1

Table 2. Average values ($\bar{X}$) and standard deviations (σ) of protein content (P%) and Zeleny sedimentation volume (ZEL) (ml), and correlation coefficients (r) between P% and ZEL of the two set of data 85B2 and 86B2

	P%		ZEL		Corr. P% - ZEL
	$\bar{X}$	σ	$\bar{X}$	σ	r
85B2	13.1	0.7	47	8.4	0.443 **
86B2	13.7	1.0	57	11.8	0.744 ***
	***		***		

* $0.01 < p < 0.05$ ** $0.001 < p < 0.01$ *** $p < 0.001$

For 85B2, data analyses equivalent to analysis 3 were carried out using extensogram resistance after 90 min (Ex 90 R) and extensogram extensibility after 90 min (Ex 90 E) as the y-variables; data analyses nos. 10 and 11, respectively.

The proportion of the variance in the y-variables explained by the PLS regression analyses, «the explained variance in y», is given for analyses 1-7. The explained variance in y represents the fit between the observed y-value and the y-value estimated from the regression analysis as a total of all the samples.

The results from the individual Cross-Validation segments are given for PLS analysis 3 carried out on 85B2 and 86B2 separately. The fit between the observed y-value and the y-value predicted from the calibration model is thus given for samples that are excluded from the calibration. As a total of all the Cross-Validation segments this is termed «the predicted variance in y». The predicted variance in y is given for data analyses nos. 8 and 9 in which all the breeding lines and all the present information about the storage proteins are included.

RESULTS

Variation in protein content and Zeleny sedimentation volume

The average protein content (P%) and Zeleny sedimentation volume (ZEL) were significantly higher in 86B2 than in 85B2 (Table 2). The correlation between P% and ZEL was significant in both years (Table 2), but the correlation coefficient between the two parameters was higher in 86B2 than in 85B2. The standard deviations in both P% and ZEL were also higher in 86B2 than in 85B2.

One-dimensional gel electrophoresis of gliadins and HMW glutenin subunits

Electrophoretic patterns (APAGE) of the gliadins for 10 breeding lines are shown in Fig. 3. The regions of the gel con-

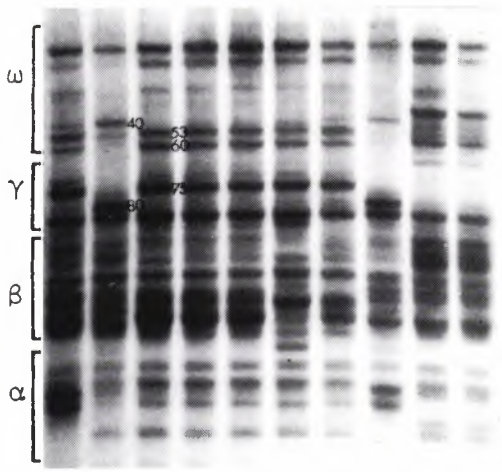

Figure 3. APAGE pattern of gliadins for 10 breeding lines from 86B2. The areas of the gel containing ω-, γ-, β-, and α-gliadins, and the variable numbers of some gliadin bands, are marked

taining the ω-, γ-, β- and α-gliadins are marked on the figure. Fig. 4 shows densitometer scans for two breeding lines, one from 85B2 and one from 86B2. More bands were resolved by the electrophoresis of 86B2 than by the electrophoresis of 85B2. The three gliadin peaks used as references for the longitudinal adjustments of the scans are marked with arrows. Digitized curves for two breeding lines from 85B2 and two from 86B2 adjusted for longitudinal extension and background staining are given in Fig. 5.

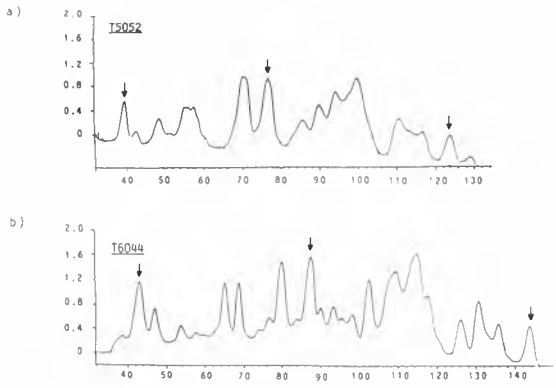

Figure 4. Densitometer scan of gliadins for two wheat breeding lines separated by APAGE; one from 85B2 (a) and one from 86B2 (b). The three peaks marked on the curve are used as references for adjustments of the curves

Densitometer scans of the HMW glutenin subunits for three varieties are shown in Fig. 6. The frequencies of different combinations of HMW glutenin subunits occurring in the material analysed are listed in Table 3. The combination 2*, 7+8 and 5+10 and the combination 2*, 13+16 and 5+10 were found to be of highest frequency. Subunits 17+18 and the null-allele were found to be rare in this material. Some of

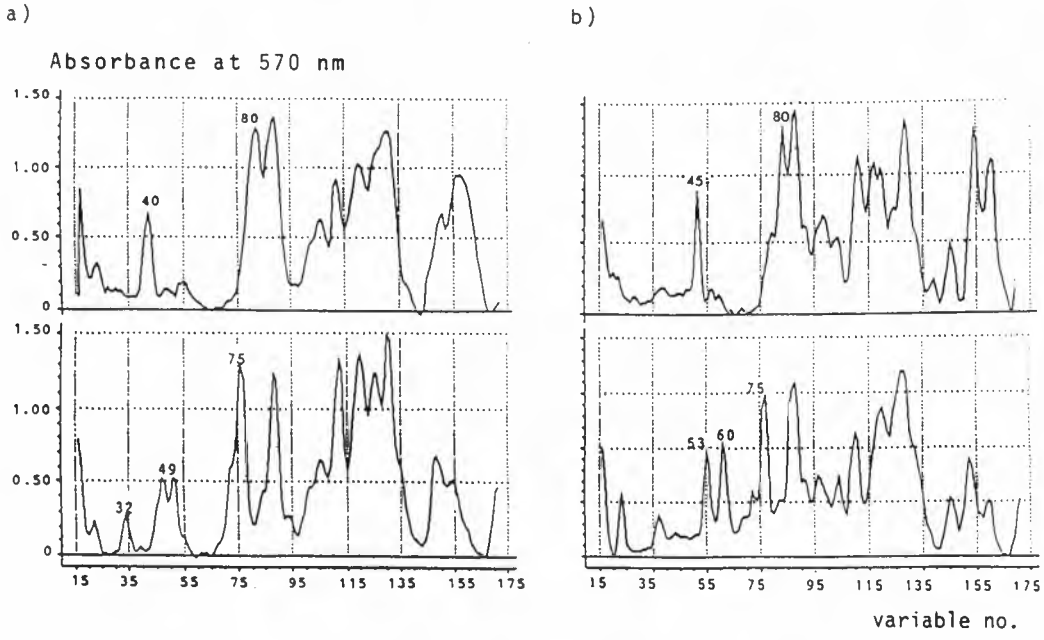

Figure 5. Adjusted digitalized scanning curve of the gliadins for two breeding lines from 85B2 (a) and two from 86B2 (b). The variable numbers of some gliadin peaks are marked on the curves

Table 3. The number of breeding lines with the various combinations of HMW glutenin subunits found in 85B2 and 86B2

85B2 HMW SUBUNITS OF GLUTENIN			NUMBER	86B2 HMW SUBUNITS OF GLUTENIN			NUMBER
2*	13+16	2+12	1	2*	6+8	2+12	1
2*	6+8	2+12	1	1	6+8	2+12	1
1	6+8	2+12	1	2*	7	2+12	1
2*	7+8	2+12	4	1/2*	7	2+12	1
2*	13+16	5+10	17	2*	7+9	2+12	1
2*	17+18	5+10	1	2*	7+8	2+12	3
2*	6+8	5+10	5	2*	13+16	5+10	7
2*	6+8/13+16	5+10	1	1	13+16	5+10	2
1	7	5+10	1	0	17+18	5+10	1
2*	7+9	5+10	1	2*	17+18	5+10	1
1	7+9	5+10	2	2*	6+8	5+10	5
1/2*	7+9	5+10	1	1	6+8	5+10	2
2*	7+8	5+10	6	2*	7	5+10	3
1	7+8	5+10	4	1	7	5+10	1
2*	13+16	5+10/2+12	1	2*	7/13+16	5+10	2
2*	6+8	5+10/2+12	2	2*	7+9	5+10	2
1	7	5+10/2+12	1	1	7+9	5+10	2
2*	7+8/6+8	5+10/2+12	1	2*	7+8	5+10	5
				1/2*	7+8	5+10	1
				0	7+8/17+18	5+10	1
				2*	7+8/17+18	5+10	2
				2*	7+8	5+10/2+12	1

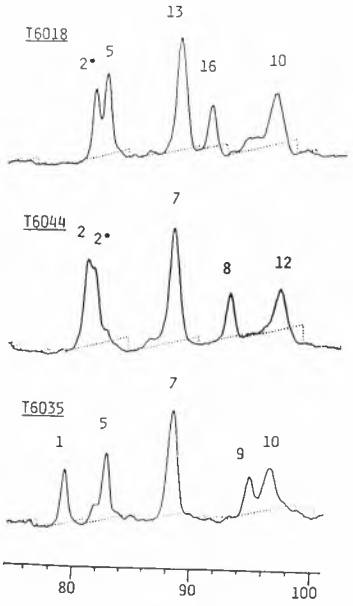

Figure 6. Densitometer scan of HMW glutenin subunits for three breeding lines. The different subunit peaks are identified according to the system of Payne & Lawrence (1983)

the breeding lines contained two sets of subunits encoded from the same locus. This is due either to some heterozygosity in the lines, or to contamination of other breeding lines in the grain samples.

Data analyses

The proportions of the variance in ZEL explained by the x-variables in PLS regression analyses nos. 1, 2 and 3 (Table 1) are shown in Fig. 7. For 85B2, 17% of the variance in ZEL was explained by the variance in P% (1). When HMW GLU (A) was included (2), 51% of the y-variance was explained, and when GLI-SC was included as well (3), the explained variance in ZEL increased to 73%.

For 86B2, the variance in P% alone (1) accounted for as much as 54% of the variance in ZEL. The proportion of the variance in ZEL explained by the regression analysis increased to 74% when HMW GLU (A) was included (2) and to 88% by further inclusion of GLI-SC (3).

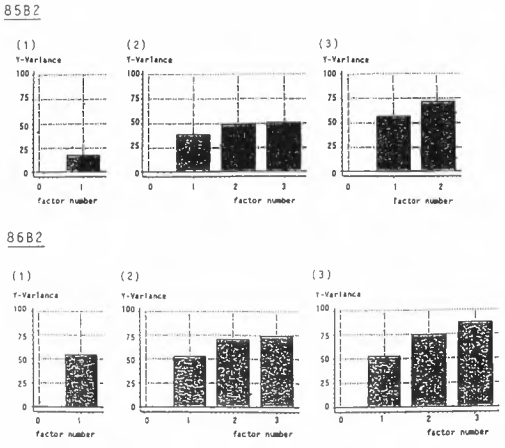

Figure 7. Percent explained variance in ZEL (the y-variable) by the x-variables in the PLS regression analyses 1, 2 and 3 for 85B2 and 86B2. The first column illustrates the proportion of the variance in y explained by the first PLS factor, the second illustrates the explained variance in y when the first two PLS factors are included, and the third when the first three PLS factors are included. The number of factors included was determined by a Cross-Validation test

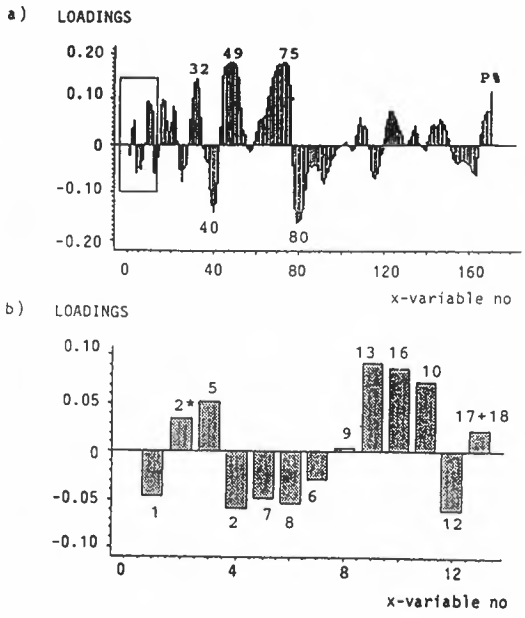

Figure 8. The «loadings» of the x-variables for the first PLS factor in the PLS regression analysis no. 3 for 85B2. The loadings of the HMW glutenin subunits are marked by a rectangle in Fig. a) and enlarged in Fig. b). The variable number of predominating gliadin bands are marked on Fig. a)

A plot of loadings of the different x-variables for the first PLS factors can visually reveal which of the x-variables that correlate with high values of the y-variable. The loadings of the x-variables for the first PLS factor in analysis no. 3 for 85B2 are given in Fig. 8. This first PLS-factor explained 58% of the variance in ZEL. It contained strong positive loadings for P% and the HMW glutenin subunits 5+10 and 13+16. The loadings for subunits 2+12, which are the alternative to 5+10, were strongly negative. In addition, some of the gliadin bands had strong positive loadings and some had strong negative loadings. These gliadin bands are marked by their identification number in Fig. 8. They comprise gliadin bands 32, 49 and 75 of strong positive loadings and gliadin bands 40 and 80 of strong negative loadings. These gliadin bands are also marked on the adjusted, digitalized scanning curves given in Fig. 5a. It can be seen from the scanning curve (Fig. 5a) that gliadin band 49 consists of at least two gliadins.

Gliadin bands that appeared to have a strong influence on bread-making quality in the 85B2 material were also found to be of importance in the 86B2 material (Fig. 9). The loadings of gliadin band 75 were strongly positive, while the loadings of gliadin band 80 were strongly negative. In addition, gliadin bands 35, 53 and 60 had strong positive loadings and gliadin band 45 strong negative loadings in this material. On examining the APAGE gels, it became evident that gliadin bands 53 and 60 correspond to band 49 of the 85B2 material (see Fig. 5a and b). Because of the slightly longer running time of the electrophoresis carried out for the 86B2 material, band 49 was separated into two bands in 86B2, giving one peak at position 53 and one at

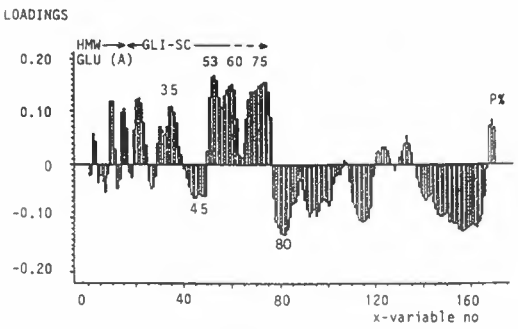

Figure 9. «Loadings» of the x-variables for the first PLS factor in PLS regression analysis no. 3 for 86B2. The variable numbers of predominating gliadin bands are marked on the figure

position 60 on the adjusted scanning curve. Similarly, gliadin bands 35 and 45 of the 86B2 material correspond to gliadin bands 32 and 40 in 85B2. Gliadin bands 35, 45, 53, 60, 75 and 80 are marked in Fig. 3.

The additional PLS factors included in analyses nos. 2 and 3 (see Fig. 7) consisted of loadings with high positive values for P% and for the HMW glutenin subunits 5+10 and 13+16. No major additional information on the gliadin scan was observed in these factors.

The explained variances in SZEL for data analyses nos. 4 to 7 are given in Table 4. When 85B2 was analysed, HMW GLU (A) alone explained 36%, while HMW GLU (A) + GLI-SC explained 42% of the variance in SZEL. For 86B2, HMW GLU (A) alone explained 17% of the SZEL variance, while HMW GLU (A) + GLI-SC explained 49%. The loadings of the electrophoretic variables for the first PLS factor in these analyses were similar to those found for data analyses nos. 2 and 3 in which ZEL was used as a y-variable and P% was included as an x-variable.

Table 4 also gives the results from the qualitative data analyses in which the electrophoretic patterns were expressed as 0-1 variables. For 85B2, the explained variance in SZEL was the same

Table 4. The explained variance in SZEL for the PLS analyses 4, 5, 6 and 7. One PLS factor is included in all the analyses as determined by Cross Validation tests

	Quantitative data analyses		Qualitative data analyses	
Dataset	(4)	(5)	(6)	(7)
85B2	36%	42%	36%	42%
86B2	17%	49%	20%	40%

in the qualitative analyses (6 and 7) as when the densitometer scans were included (analyses nos. 4 and 5). Thus, for this dataset, the relevant information on both protein groups for predicting SZEL appears to be related to the presence or absence of the various HMW glutenin subunits and six gliadin bands. For 86B2, however, inclusion of the gliadin scan explained slightly more of the variance in SZEL than just the presence or absence of the six gliadin bands.

The predictive validity of the calibration model obtained for analysis no. 3 is given in Fig. 10a for 85B2 and in Fig. 10b for 86B2. ZEL was predicted from all the x-variables in these analyses. Each curve represents the result from one Cross-Validation segment consisting of

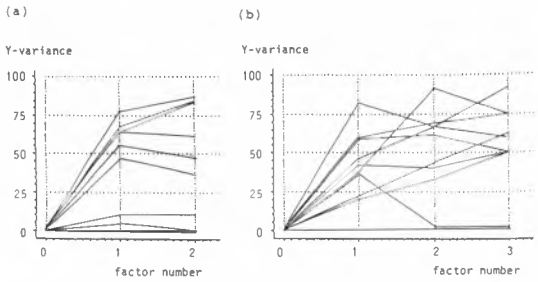

Figure 10. Predicted variance in ZEL for 85B2 (a) and 86B2 b). The curves represent, cumulatively, the predicted variance in y, for each of the Cross-Validation segments, by increased numbers of PLS factors included in the calibration model. The results from the different Cross-Validation segments is scaled to unit variance

five or six breeding lines. The predicted sedimentation volume fits the observed value for most of the Cross-Validation segments relatively well, but for some segments it failed. This phenomenon was also observed for the other data analyses carried out on each year separately. In particular, prediction of SZEL failed for many breeding lines in the 86B2 material when this quality parameter was predicted from the HMW glutenin subunits only (data analyses nos. 4 and 6, results not shown).

The predicted variance in ZEL and SZEL for the combined analyses of 85B2 and 86B2 (analyses 8 and 9, respectively) is given in Fig. 11. All Cross-Validation segments summarized indicated that 60% of the variance in ZEL can be predicted for new samples when P%, HMW GLU (A) and GLI-SC are known. For SZEL, the Cross-Validation test indicated that 35% of the variance can be predicted from HMW GLU (A) and GLI-SC.

Loading plots of the x-variables for the first PLS factors in analyses 8 and 9 gave the same information as similar analyses carried out for each year separately (see loading plots for prediction of ZEL in Fig. 8 and 9).

Regression coefficients for SZEL prediction estimated from the first two PLS factors in analysis 9 are given in Fig. 12. The regression coefficients are shown for the 13 HMW GLU (A) variables and

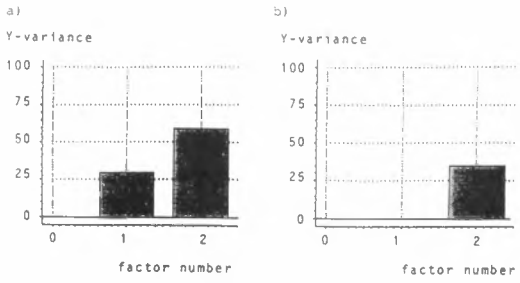

Figure 11. Predicted variance in ZEL (a) and SZEL (b) for PLS analyses nos. 8 and 9 respectively, evaluated by Cross-Validation test

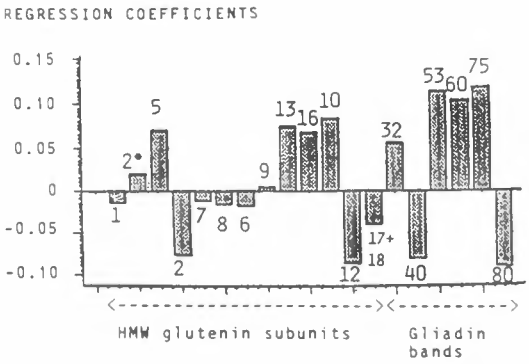

Figure 12. Regression coefficients for prediction of SZEL estimated from the first two PLS factors in PLS analysis 9. The HMW glutenin subunits are numbered according to the system of Payne & Lawrence (1983), and the six gliadin bands by their variable numbers

gliadin bands 32, 40, 53, 60, 75 and 80. Most important among the HMW glutenin subunits were the subunits 5+10 and 13+16 having positive regression coefficients, and subunits 2+12 which had negative regression coefficients. In addition, gliadin bands 53, 60 and 75 had strong positive regression coefficients, whereas those for gliadin bands 40 and 80 were strongly negative.

For 85B2, the relationship between electrophoretic data and extensogram resistance and extensibility was analysed in data analyses 10 and 11, respectively. The loadings of the x-variables for the first PLS factor, explaining 47% of the y-variance in both analyses, are given in Fig. 13. These loadings revealed basically the same information about the associations between the different electrophoretic bands and bread-making quality as found for ZEL.

Two-dimensional electrophoresis of gliadins

The two-dimensional electrophoresis (APAGE*SDS-PAGE) of one breeding line, T8020, which contains the gliadin bands found to be associated with good bread-making quality, is shown in Fig.

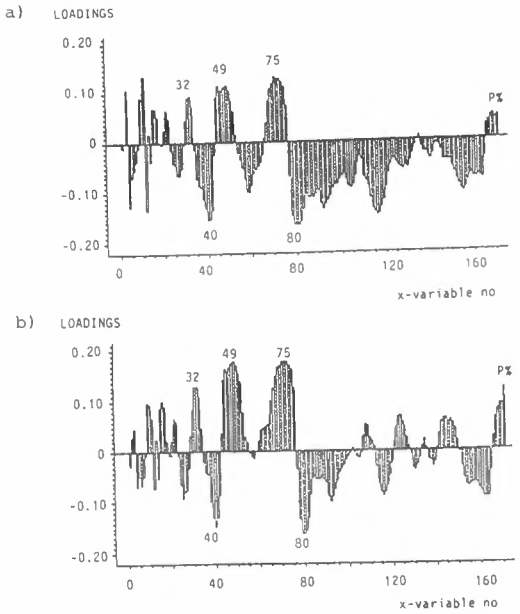

Figure 13. The «loadings» of the x-variables for the first PLS factor in data analyses 10 (Fig. a) and 11 (figure b), in which extensogram resistance and extensibility, respectively, were used as y-variables. The variable numbers of predominating gliadin bands are marked on the figure

14. The major γ-gliadin, marked with a double-headed arrow corresponds to the gliadin band 75 (on APAGE). The ω-gliadin, marked with a filled arrow, corresponds to gliadin band 60, and the two ω-gliadins marked with open arrows corresponds to gliadin band 53. The ω-gliadins corresponding to gliadin band 32 are not easily identified. One or more of the three spots marked with thin arrows may move into this band when subjected to APAGE.

DISCUSSION

The relationship between the composition of gliadins and HMW glutenin subunits and bread-making quality has been examined in this study. The bread-making quality has been tested by means of Zeleny sedimentation volumes and extensograms which analyse properties of the proteins important for bread-making quality.

The results revealed that variation in gliadins as well as in HMW glutenin subunits contributed considerably to the variation in Zeleny sedimentation volume. The effects of the various HMW subunits of glutenin on the quality tests were in accordance with the results of Uhlen (1990a). In addition, several gliadin bands were found to be strongly associated with bread-making quality. Gliadin bands 32, 53, 60 and 75 were positively associated with the Zeleny sedimentation volume and with the resistance and the extensibility of the extensogram, whereas gliadin bands 40 and 80 were negatively associated with these quality parameters. Similar quality associations for these gliadin bands were found when another set of 25 breeding lines was analysed (Uhlen et al. 1987).

The breeding line T8020, having the gliadin bands positively associated with bread-making quality, is reported by Sontag (unpublished results) to contain the gliadin blocks Gld1A2, Gld1B8 and Gld1D3 according to the nomenclature of Metakovsky (unpublished results) given in Fig. 2. According to this information and to the chromosomal location of the ω- and γ-gliadins given for the same breeding line on the two-dimensional gel (Fig. 14), it is concluded that the gliadin bands 75, 60 and 53 correspond to the gliadins of block Gld1B8. Band 53 may also contain a ω-gliadin belonging to block Gld1A2, when this block is present in addition to Gld1B8. Furthermore, one of the three ω-gliadins that possibly correspond to gliadin band 32 (marked with thin arrows in Fig. 14) belongs to Gld1A2. The chromosomal location of the genes encoding the other two ω-gliadins are at present not known. It was observed in the present material that gliadin bands 75, 60 and 53 always occurred together, supporting the conclusion that

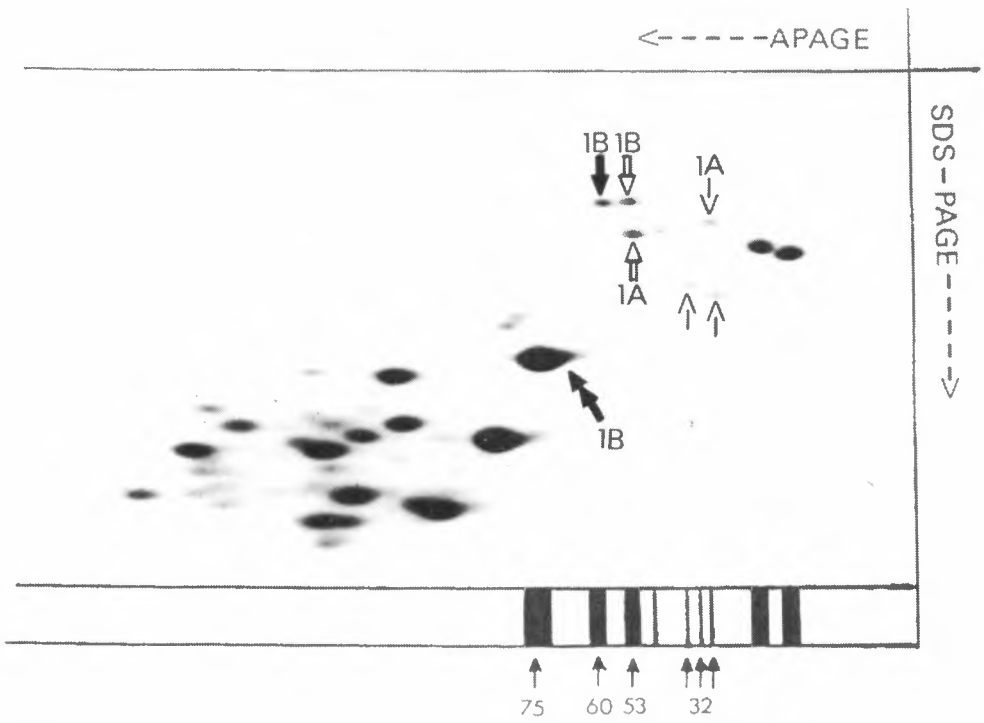

Figure 14. Two-dimensional electrophoresis (APAGE*SDS-PAGE) of the gliadins of the breeding line T8020. The arrows indicate the gliadin spots corresponding to the gliadin bands at variables 32 (thin arrows), 53 (open arrows), 60 (filled arrow) and 75 (doubleheaded arrow). The corresponding electrophoretic pattern of one-dimensional APAGE is illustrated by projecting the gliadin spots in the vertical direction. The number of the chromosomes (1A, 1B), from which some of these gliadins are encoded is marked on the figure

these gliadin bands belong to one gliadin block. Similarly, gliadin bands 40 and 80, negatively associated with quality, occurred together, and were alternative to gliadin bands 75, 60 and 53. From these observations and according to the identified gliadin blocks of Metakovsky (unpublished results) it is concluded that bands 40 and 80 also belong to gliadin blocks encoded by chromosome 1B.

None of the gliadin bands from the region of the one-dimensional gel with extensive overlap (the region containing α- and β-gliadins) were related to quality in this study. It is possible that other techniques giving a better separation of the gliadins (i.e. two-dimensional electrophoretic techniques, RP-HPLC) may reveal quality associations for these gliadins.

When a calibration model was made based on protein content, the HMW glutenin subunits and the six above-mentioned gliadin bands, the bread-making quality was well predicted for most breeding lines, whereas for some breeding lines the calibration model failed. This is most likely due to variation in storage proteins that was not included in the present calibrations. Data on the composition of LMW glutenin subunits were not included. Furthermore, one-dimensional APAGE do not separate all the gliadins, causing several gliadins or gliadin blocks to be hidden in the electrophoretic patterns. Another factor that may contribute to the prediction failure is the distorted distribution of the storage protein subunits in this material, as seen from the distri-

bution of the HMW glutenin subunits (Table 3).

Most of the variation found to be relevant for predicting the quality tests appeared to be related to the presence or absence of the various HMW glutenin subunits and six gliadin bands. The quantitative differences measured by densitometric scanning did not give consistent additional predictive information. For well-separated HMW subunits of glutenin, densitometer scanning of the bands has been reported as an accurate method for quantitative measurements (Uhlen & Ringlund 1987). However, by means of this technique, Payne et al. (1981b) and also Uhlen (1990b) found that the relative intensities of different HMW glutenin subunit bands are approximately constant from one variety to the other. As a consequence, the presence or absence of the various HMW glutenin subunits along with the protein content contain most of the variation present in the densitometer scan of these subunits.

For the gliadins, inclusion of the whole scanning curve in the PLS regression analyses made it possible to reveal quality associations for six gliadin bands of the ω- and γ-types. Since these gliadin bands appeared to be strongly associated with bread-making quality, they may be used as markers for bread-making quality in wheat breeding programmes. As the genes for the ω- and γ-gliadins are closely linked to the genes for the LMW subunits of glutenin, it is not known which of these two storage protein groups is responsible for the observed differences in quality.

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MODELLING PHENOTYPIC VARIATION IN PLANTS

Single locus models without genotype by environment interactions

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The models presented describe phenotypic variation associated with genetic variation at a single locus for diploids and autotetraploids. The implications of the model assumptions are described in some detail. Values for parameters describing genetic effects and environmental effects are set throughout in relation to the additive genetic effect. This allows calculation of expected heritabilities for any model situation, and also a general aproach in distinguishing between discontinuous variation (i.e. qualitative characters) and continuous variation (i.e. quantitative characters).

Key words: Genotype, phenotype, quantitative characters

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In principle, genotypic variation is discontinuous in the sense that differences between genotypes are in units of one allele, and not in a continuum of fractions of alleles. Phenotypic variation, however, may be discontinuous or continuous, depending on the relative influence of environmental effects on the phenotypic expression, and on the scale used to assess the phenotype.

For the discontinuous variation, few loci are often assumed, and that their genes have "large" effects. For a character with a continuous phenotypic variation, i.e. a quantitative character, a normal distribution is often assumed, as an inference often follows the assumption that it must be governed by many loci. The onstart may also be the other way around: because the studied character is a complex one, it must be governed by many loci, and therefore it is reasonable to assume a (near) normal phenotypic distribution. Both viewpoints stem from the known relationship between the normal- and the binomial distribution. In order to explain a (near) normal phenotypic distribution as being a consequence of a binomial genotypic distribution, the assumption of many loci with small and near equal gene effects is so frequently adopted that this appears more or less as the "household" assumption for quantitative characters. For genetic interpretation of statistical parameters in certain experiments, the latter assumption is ne-

cessary, but not always so. In any case, a normally distributed environmental effect, or error element, is assumed to be overlaid the genotypic distribution.

However, many published observations of phenotypic distributions for quantitative characters in plants seem to deviate from normality in one or more ways (Beddows 1969, Honne 1979a). They may have two or more maxima, they may be skewed and/or kurtotic. Also, several works have been published which clearly demonstrate that gene effects for quantitative traits may be both "large" and variable among loci (see for instance Wehrhahn & Tai 1988 with references).

The aim of this paper is to describe discontinuous and continuous variation on a single locus model, and to indicate possible criteria for making a distinction between the two types. Some cases where the principal model is tentatively fitted to describe observed phenotypic variation will also be discussed. The model adopted is more or less the standard approach, but its implications will be drawn in more detail than usual.

MODEL ASSUMPTIONS AND SPECIFICATIONS

Diploid model

We consider one locus with two alleles, A and a, in a diploid population. The relative frequency of the positive allele A is u, while that of allele a is $(1\text{-}u)=v$. The genetic effects are specified as deviations from the midpoint between the two homozygous genotypes (Fisher et al. 1932), with d representing the additive- and h the dominance effect. The genotype frequencies used are panmictic equilibrium frequencies, although generally the following approach may be used with any genotypic composition of the population, provided the genotype frequencies are known or specified.

In addition to the genetic effects, the phenotype is determined by environmental effects. These are assumed to be normally distributed with mean 0 and standard deviation σ_e. Genotypes and environmental effects are distributed independently. Although a regression type of genotype by environment interaction may be accommodated, I will assume here that there is no g by e interaction. The assumptions and specifications are summarized in Table 1.

Autotetraploid model

For the autotetraploid also I will consider the simplest case with one locus containing two alleles, A and a. Their frequencies are designated as in the diploid case. In addition to the allele frequencies, the model should also account for the probability of double reduction, designated as α. This is the probability that pieces of sister chromatides containing the locus under consideration end up in the same gamete. The parameter α^* which appears below is defined as

$$\alpha^* = \frac{3\alpha}{(2+\alpha)}$$

For the autotetraploid locus we have the following five possible genotypes, with parameters describing the genetic effects as deviations from the midpoint, m, between the nulliplex and the quadriplex genotypes.

Genotypes	Genetic effects
AAAA	$g^4 = m + 2d$
AAAa	$g^3 = m + d + 3h + 3w + x$
AAaa	$g^2 = m + 4h$
Aaaa	$g^1 = m - d + 3h - 3w + x$
aaaa	$g^0 = m - 2d$

h,w, and x specify digenic-, trigenic-, and quadrigenic interactions, respectively (Kempthorne 1957, Honne 1986). For the examples for this model I will also use panmictic equilibrium frequencies. (Deductions of these may be found in Seyffert 1960, or Honne 1979b.) The further assumptions are parallel to the

Table 1. Specifications for the phenotypic variation with a diploid one locus genetic model and a normally distributed environmental effect. No genotype by environment interaction

Environment, env. eff., frequency	Genotype, gen.eff.,freq. *AA* u^2	*Aa* $2uv$	*aa* v^2	Row Mean	Var.
$E_1, e_1, f(e_1)$	$d+e_1$	$h+e_1$	$-d+e_1$	$(u-v)d+2uvh+e_1$	V_g
$E_2, e_2, f(e_2)$	$d+e_2$	$h+e_2$	$-d+e_2$	$(u-v)d+2uvh+e_2$	V_g
. . .	.	.	.	.	.
. . .	.	.	.	.	.
$E_n, e_n, f(e_n)$	$d+e_n$	$h+e_n$	$-d+e_n$	$(u-v)d+2uvh+e_n$	V_g
Col. mean	d	h	$-d$	$(u-v)d+2uvh$	V_g
Col. var.	V_e	V_e	V_e	V_e	-

Phenotypic mean. $(u-v)d+2uvh$
Phenotypic variance: $V_g + V_e$
Variance of row means: $V_e\sigma_e^2$
Variance of column means: $V_g = 2uv(d-(u-v)h)^2+4u^2v^2h^2$

diploid case, and are summarized in Table 2. Here the genotype frequencies are given in terms of gamete frequencies assuming random mating. The gamete frequencies in terms of allele frequencies and probability of double reduction are at equilibrium: *AA*: $r=u^2+a^*uv$, *Aa*: $s=2(1-a^*)uv$, *aa*: $t=v^2+a^*uv$.

Composition of the modelled phenotypic variation

Variation within each class of genotype is caused solely by environmental variation. There is no covariance between genotype and environment and no *g* x *e* interaction (Tables 1 and 2). There are no differences between classes of genotypes conditional on environment. Variation

Table 2. Specifications for the phenotypic variation with an autotetraploid one locus genetic model and a normally distributed environmental effect. No genotype by environment interaction

Environment, env. eff., frequency	Genotype, frequency, and gen. eff. *AAAA* r^2 g^4	*AAAa* $2rs$ g^3	*AAaa* $2rt+s^2$ g^2	*Aaaa* $2st$ g^1	*aaaa* t^2 g^0	Row mean	var.
$E_1, e_1, f(e_1)$	g^4+e_1	g^3+e_1	g^2+e_1	g^1+e_1	g^0+e_1	μ_g+e_1	V_g
$E_2, e_2, f(e_2)$	g^4+e_2	g^3+e_2	g^2+e_2	g^1+e_2	g^0+e_2	μ_g+e_2	V_g
. . .	.	.	.	.	.	.	.
. . .	.	.	.	.	.	.	.
$E_n, e_n, f(e_n)$	g^4+e_n	g^3+e_n	g^2+e_n	g^1+e_n	g^0+e_n	μ_g+e_n	V_g
Col. mean	g^4	g^3	g^2	g^1	g^0	μ_g	V_g
Col. var.	V_e	V_e	V_e	V_e	V_e	V_e	-

Phenotypic mean: $2(u-v)d+2(4uv+s)h+6s(u-v)w+2s(1-s)x = \mu_g$
Variance of row means: $V_e=\sigma_e^2$ (= variance within class of genotype.)
Variance of col. means: V_g (= variance between classes of genotype.)
Phenotypic variance : $V_g + V_e$

among classes of genotypes is entirely genetic.

The probability of an environmental effect in the interval from x to z is given by:

$$f(e_i) = \int_x^z N(0,\sigma_e)de$$

where $N(0,\sigma_e)$ means a normal density function with mean = 0, and standard deviation = σ_e.

A particular class of phenotypes, P_i, within a genotypic class, G_j, is given by:

$$f(P_{i,j}) = \left\{ \int_a^b N(G_j,\sigma_e)dP \right\} f(G_j)$$

where *a* and *b* are the lower and upper phenotypic class limits, $N(G_j,\sigma_e)$ means a normal density function with mean G_j = genotypic value of the actual class of genotypes; $f(G_j)$ designates the relative frequency within the population of that genotypic class. The total frequency of the particular phenotypic class in the population is then totalled over all genotypic classes contributing that phenotype. This is given by:

$$f(P_i) = \sum_{j=1}^{k} \left\{ \int_a^b N(G_j,\sigma_e)dP \right\} f(G_j)$$

where G_j, j = 1,2, ..,k refers to the k genotype-classes contributing phenotypes of the actual class, P_i.

The phenotypic standard deviation within a genotypic class is σ_e, that is to say, equal to the total environmental standard deviation in the population. The contribution of the particular class to the total environmental variation within the population is thus $\sigma_e^2 = V_e$ weighted with the genotypic class frequency. It is only the phenotype frequency within the population which is weighted with the genotype class frequency. The variance within each class of genotypes is unaffected by the size of the class. (The variances here are the results of predefined effects of genotype and environment respectively, and not estimates based on random samples of these effects.)

GRAPHIC ILLUSTRATIONS OF THE MODEL

Several examples with various parameter values for the diploid model are shown in Figs. 1-3. Note that both the non-additive gene effect and the standard deviation of the environmental effects are defined in relation to the additive gene effect, *d*. These relations allow us to identify the necessary criteria in distinguishing between continuous and discontinuous phenotypic variation, and to calculate expected heritabilities for the various model situations.

Note also that the ordinate of the density function is plotted as $y \cdot \sigma_e$ (y is the usual ordinate of the normal density function). That is to say, the ordinate of the density function is comparable to ordinates of a histogram with class width equal to σ_e, and the area under the compound density function is also equal to σ_e. For area of unit *d*, the ordinates of the graphs in columns 1 and 2 in Figs. 1-6 must be divided by 0.25 and 0.5, respectively.

For a normal density function more than 99.7% of the observations are expected within $\mu \pm 3 \cdot \sigma$, and more than 95% within $\mu \pm 2 \cdot \sigma$. It is clear then that when the distance between two consecutive classes of genotypes is approximately equal to or exceeds $6 \cdot \sigma_e$, the phenotypic distribution is expected to be discontinuous. When the distance is less than $6 \cdot \sigma_e$, the distribution is expected to be continuous. For the diploid model the largest distance between two classes is $(d + |h|)$, with an upper limit of $2d$. It may therefore be said that when $6 \cdot \sigma_e \leq (d + |h|)$, we will have a qualitative character; while if $6 \cdot \sigma_e > (d + |h|)$, we will

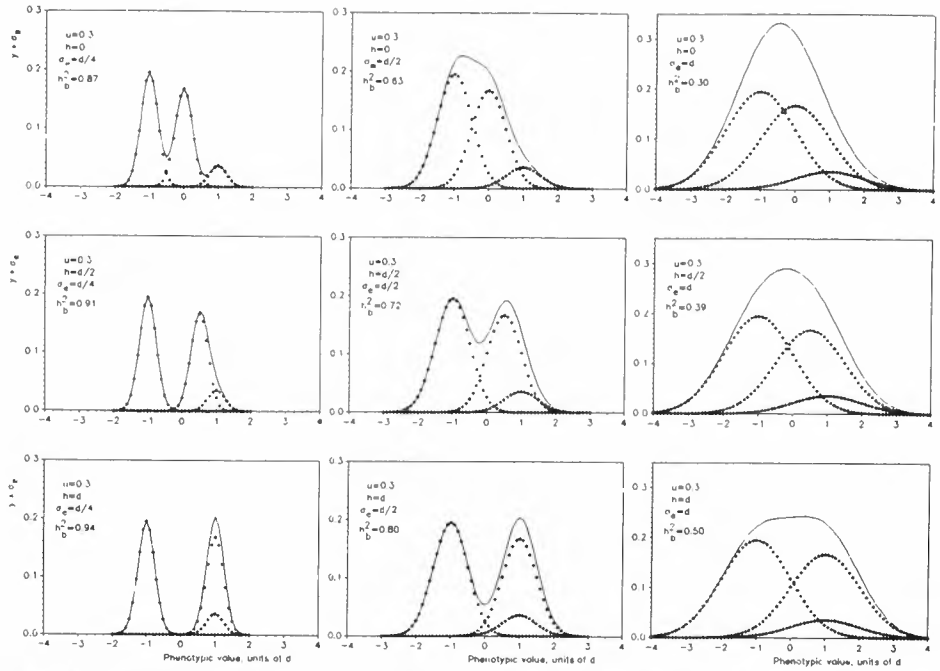

Fig. 1. Diploid model. Illustration of the composite phenotypic distribution (density function) when the degree of dominance changes from no dominance (top row) to partial dominance (middle row) to complete dominance (bottom row). The environmental standard deviation increases among columns from left to right. The expected composite phenotypic distribution is shown by the continuous line; phenotypic distribution of each of the genotype classes is indicated by the dotted lines. Frequency of the positive allele is $u = 0.3$

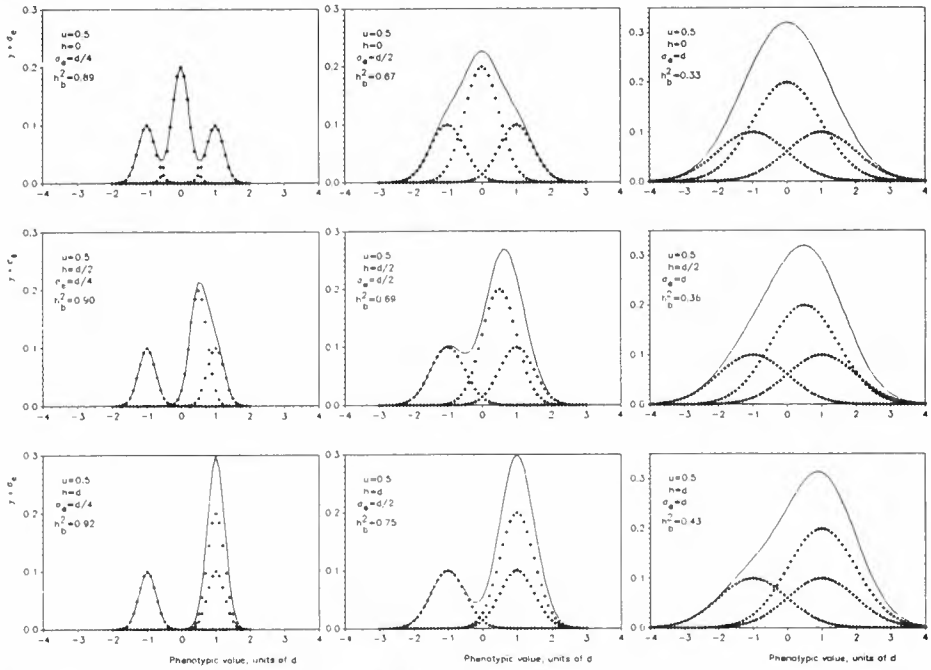

Fig. 2. Diploid model, $u = 0.5$, otherwise similar to Fig. 1

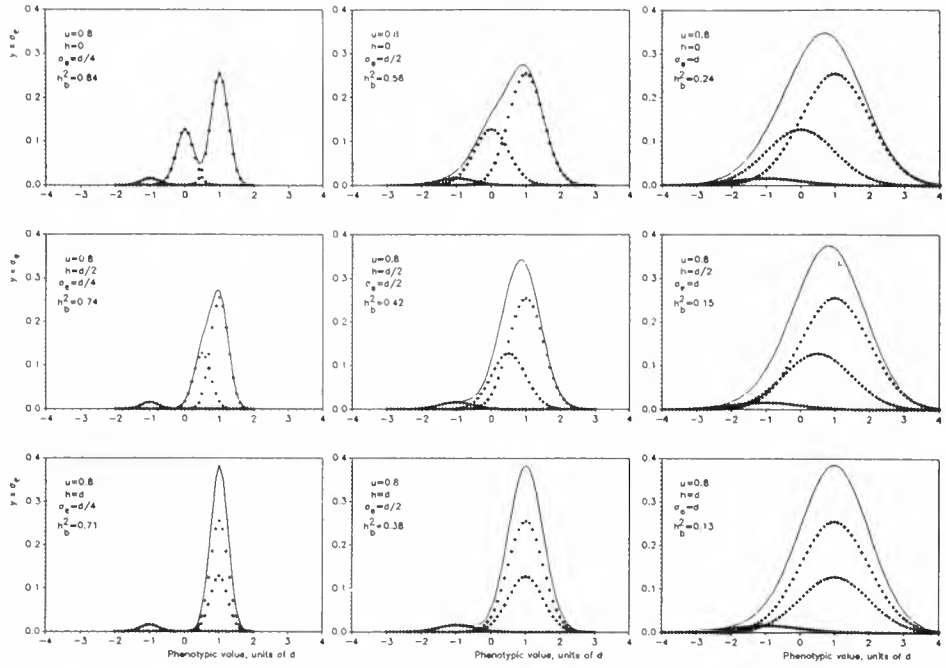

Fig. 3. Diploid model, $u = 0.8$, otherwise similar to Fig. 1

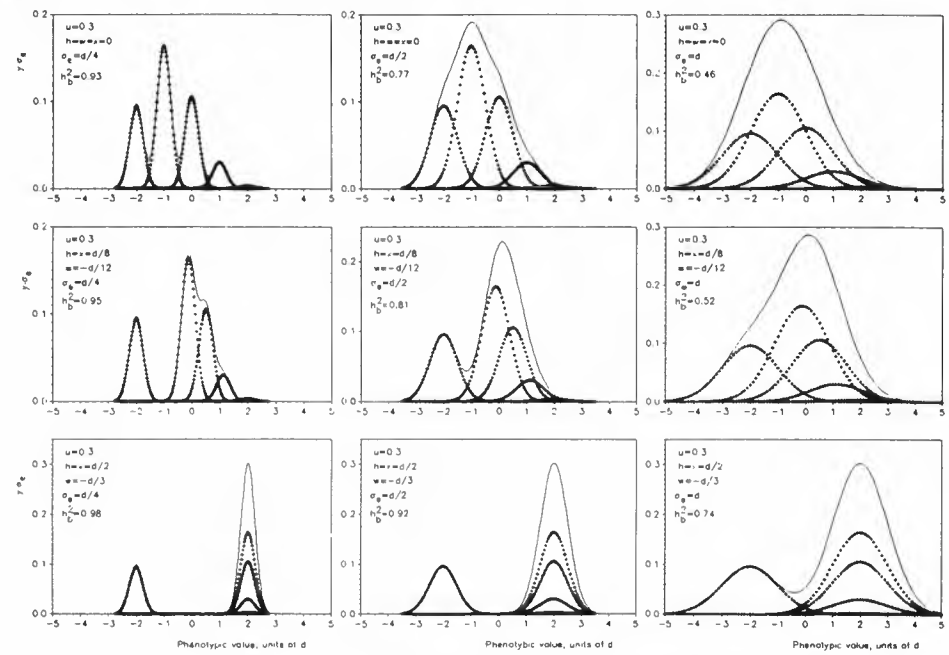

Fig. 4. Autotetraploid model, $u = 0.3$. Five genotypic classes. Otherwise similar to Fig. 1

have a quantitative character. For practical purposes, considering also sample size, we may expect borderline cases when the largest distance is between say 4 to 6 times σ_e.

For the autotetraploid model, illustrated in Figs. 4-6, the largest distance between two consecutive classes will usually be found between the nulliplex and simplex, or between the triplex and quadriplex, depending on the direction of non-additive gene-effects. (For some parameter values, however, it may be between duplex-simplex or duplex-triplex.) (Sets of likely parameter values for autotetraploids have been discussed by Opsahl 1964, Gallais 1977, Honne 1981, 1986.) For the largest distances of the first category, the distance will be $Dc = (d + |3h| + |3w| + |x|)$, with an upper limit of $4d$. Evidently for the autotetraploid the criterion for discontinuous variation will be $6 \cdot \sigma_e \leq Dc$, while continuous variation is expected if $6 \cdot \sigma_e > Dc$.

COMPARING THE MODEL TO SOME OBSERVATIONS OF PHENOTYPIC VARIATION

The first example is for a "classical" trait, namely plant height in peas. The original seed material for the parental lines used was obtained from the Mendel Museum in Brno, by the Department of genetics and plant breeding, Agricultural University of Norway, and later maintained at the Department.

After an initial cross between a tall and a short inbred line, a total of 172 F2 plants were grown in pots in a glasshouse. The two parental lines and a sample of F1 were grown at the same time. After flowering, the plant height was measured in centimetres, and the number of in-

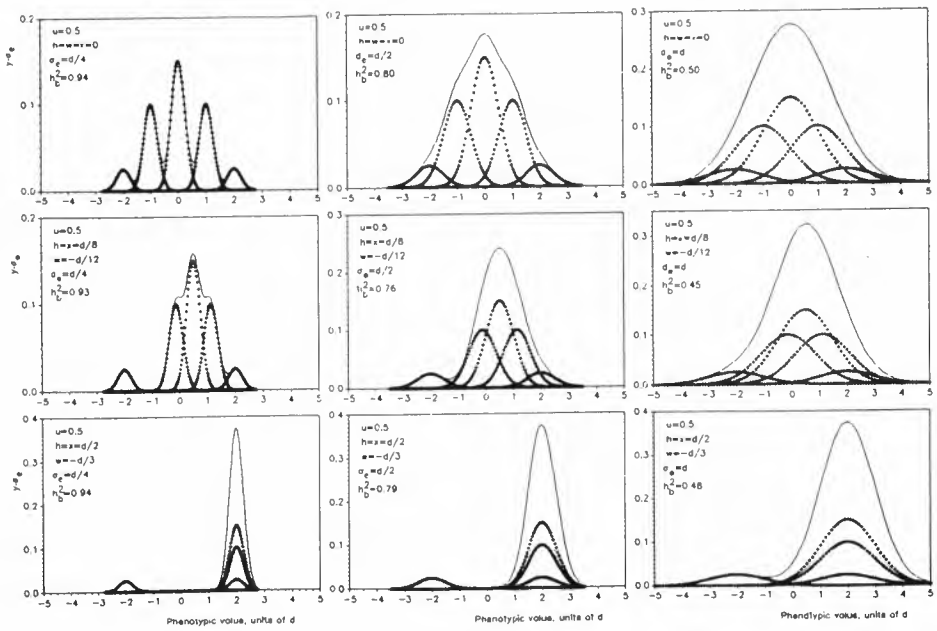

Fig. 5. Autotetraploid model, $u = 0.5$, otherwise similar to Fig. 4

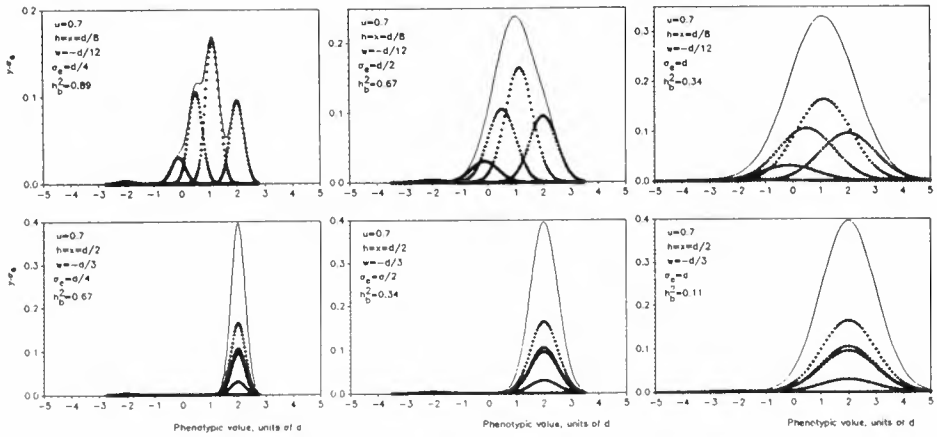

Fig. 6. Autotetraploid model, $u = 0.7$, otherwise similar to Fig. 4

ternodes counted. (The initial cross was not between isogenic lines, as it also segregated for seed colour, green vs yellow, and for smooth vs wrinkled seed coat.)

For reasons which are unknown, the short F2 plants were considerably shorter than the short parental line. Also the number of internodes per plant changed from parental lines to F1 and to F2 in a pattern not consistent with expectation for a single locus with dominance. The parameters of the fitted phenotypic model are therefore not the ones estimated from linear combinations of P1, P2, and F1 means, but somewhat modified to account for the discrepancies mentioned.

An additional problem as compared with the principal model is the large span of the phenotypic values on the scale used. The tallest phenotypes are an order of magnitude taller than the shortest. This makes it doubtful that the environmental variance intra class of genotypes is the same. Indeed, the tall parental line had significantly higher phenotypic variance than the short line. The intra class phenotypic standard deviation used for the model therefore increases from the short class ($\sigma_{e1} = 16$) to the two taller classes ($\sigma_{e2} = 20$). In Table 3 parameters of the model are compared with estimates

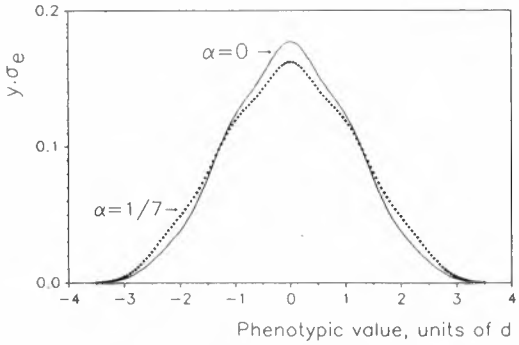

Fig. 7. Effect of double reduction when $u = 0.5$, $\sigma_e = d/2$, and $h = w = x = 0$. Heritability is 0.80 when $\alpha = 0$, and 0.83 when $\alpha = 1/7$

and predictions for F2 based on P1, P2, and F1, and with estimates from F2.

A graphic illustration of the observed phenotypic distribution and the fitted model is shown in Fig. 8a. From this figure, and from the last two lines of Table 3, it will be seen that a discontinuous phenotypic distribution, or rather a borderline case, is expected. The discontinuity is «hidden» by the grouping of observations in the histogram, but a "gap" of 19 cm

Table 3. Parameters of the model for phenotypic distribution of plant height in cm, compared with estimates from F_2, and with estimates based on P_1,P_2, and F_1 (see Fig. 8)

Parameter	Model	F2	P1,P2,F1
F_2 mean	122.5	120.8	121.6
m (midpoint)	105.0	-	110.6
d	65.0	65.8	50.8
h	35.0	-	22.0
h/d	0.53	-	0.43
s_e (weighted)	19.0	-	19.9
Vg	2418.8	2272.9	1412.6
h_b^2	0.86	0.84	0.77
$(d+h)$	100.0	-	72.8
$3\cdot\sigma_{e1}+3\cdot\sigma_{e2}$	108.0	-	119.4

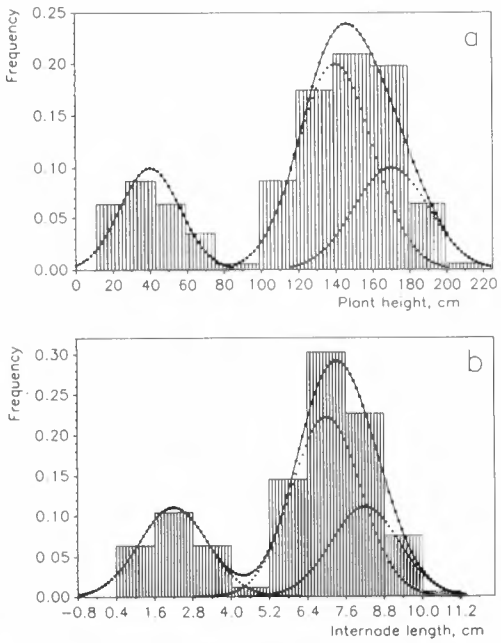

Fig. 8. Phenotypic distribution of 142 F_2 plants in peas. Histogram: observations grouped; continuous line with filled squares: model expectations with ordinate fitted to the class width. a: Plant height in cm. Note different class width for the tall and short groups (see Table 3 and text). b: Internode length in cm, class width 1.2 cm.

with no observations occurred between the short and the tall groups.

Evidently, on the short vs tall scale, the character is observed as with complete dominance for tall plants. On the centimetre scale, plant height is observed as with incomplete dominance, h/d around 1/2, but with a practically discontinuous phenotypic distribution.

If the observed "gap" is taken as "true", the 172 observed F_2 plants segregated into 128 tall vs 44 short, while the expected 3:1 is 129:43. (Which again results in a suspicously low Chi-square of 0.0311.) None of the observations have been discarded. The fitted model deviates from the grouped observations with a non-significant Chi-square of 2.80 (7 df, $P \approx 0.9$).

As mentioned above, the number of internodes was counted, and their average length calculated. A model fitted to the grouped observations of F_2 is shown in Fig. 8b. Neither the fitted model nor observations suggests a "gap" in the phenotypic distribution. For the fitted model we have $(d+h) = 4.8\cdot\sigma_e$, which suggests a continuous phenotypic distribution. Chi-square for goodness-of-fit is not significant.

The next example is from a diploid outbreeding species, namely *Festuca pratensis*. The observations are heading date for 200 randomly selected clones from the variety 'Løken'. The clones were planted in a field trial in plots of four ramets, and in a randomized block design with two replicates. As there are no possible estimates of gene frequencies or genetic effects in the present experiment, their parameter values in the model are guesswork, although it may be called "qualified guesswork" (cf. estimates by Simonsen 1977). The model is in this case an attempt to describe the observed phenotypic distribution with as simple a model as possible.

The tentative model fitted to a histogram of the observations is shown in Fig. 9. The observations are significantly skewed ($g1 = 0.694$, $P < .01$). A summary of

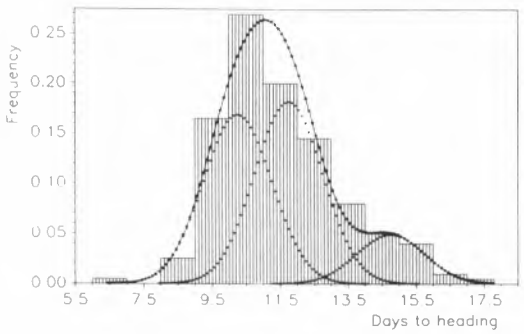

Fig. 9. Phenotypic distribution of days to heading of 200 randomly selected clones of *Festuca pratensis*, var. 'Løken'. Histogram: observations grouped, class width is one day. Continuous line with squares: expected phenotypic distribution with model parameters as given in Table 4.

the model parameters and expectations compared with the observations is given in Table 4. As can be seen from the figure and the table, the model adequately describes the observations.

Table 4. Observations of days from June 1 to heading for 200 randomly selected clones of *Festuca pratensis*, var. 'Løken', compared with a tentative phenotypic model. The genetic model has one locus with two alleles

Parameter	Model	Observations
m	15.50	-
d	2.25	-
h	- 0.75	-
h/d	- 1/3	-
u	0.35	-
mean	11.48	11.44
V_e	1.0	0.99
Vg	1.98	2.22
h_b^2	0.67	0.69

Goodness-of-fit: $X^2_{(7)} = 4.26$ ($P \approx 0.75$)

The final example is from the outbreeding species *Dactylis glomerata*. This species behaves cytologically as an autotetraploid (McCollum 1958, Honne 1973), and clear indications of tetrasomic inheritance have been found (Cuany 1958).

The character used here is the average diameter of the cross-section of plants grown in a field experiment with two replicates of clones in plots with five ramets. The experiment is described by Honne (1979). Data are from the populations 'Holt' (Fig. 10a) and 'Hattfjelldal' (Fig. 10b). Parameter values of the models fitted in these figures are given in Table 5, where model expectations are also compared with estimates for the grouped observations. The Chi-squares for goodness-of-fit are not significant. Again a simple one locus model is sufficient to describe the observed phenotypic variation, although there are very good reasons for assuming that at least for this character, variation in more than one locus is involved. It should be pointed out

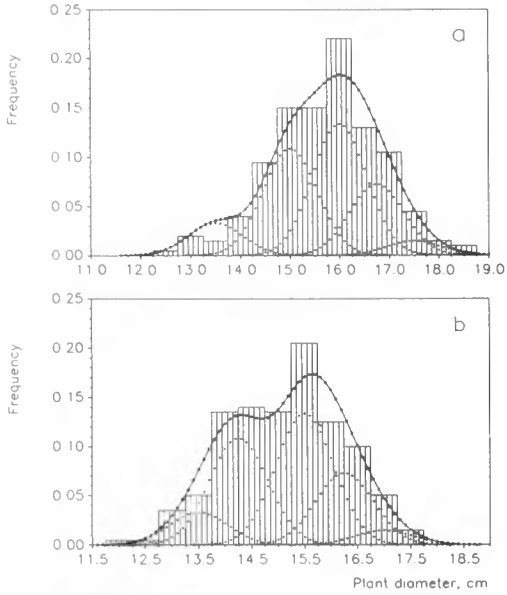

Fig. 10. Phenotypic distribution of average plant diameter. a: population 'Holt', b: population 'Hattfjelldal'. Histogram: grouped observations, class width = 0.5cm; continuous line with squares: expected phenotypic distribution with parameter values as given in Table 5; dotted curves: expected phenotypic distribution of each genotypic class of the model

Table 5. Parameter values and expectations for phenotypic model of average plant diameter, compared with estimates from the grouped observations of the character in the two populations 'Holt' and 'Hattfjelldal'

Parameter	'Holt'		'Hattfjelldal'	
	Model	Obs.	Model	Obs.
m	15.5	-	15.25	-
d	1.0	-	0.875	-
h	0.125	-	0.0625	-
w	-0.04167	-	0.04167	-
x	0	-	-0.1875	-
a	0	-	0	-
u	0.45	-	0.45	-
σ_e	0.55	-	0.55	-
mean	15.7	15.7	15.15	15.14
Vg	0.9763	1.18	1.0560	1.10
V_e	0.3025	0.40	0.3025	0.40
h_b^2	0.76	0.75	0.78	0.73

that the tendency to skewness in the two distributions is not significant at the 5% level.

Now, in all the examples where the simple model has been fitted to grouped observations, one might suspect that the fit of the model would be very dependent on how the class borders are selected. Several runs with various class borders compared to the models with parameter sets as presented show the fit of the model to be fairly robust against shifting of class borders for the grouped observations.

DISCUSSION

The models presented are in principle the same as those frequently adopted in the standard statistical approach, only their implications are shown in more detail than in many other cases. In addition, the standard deviation of the environmental effect (in experiments it may be the residual variation) is defined in relation to the additive genetic effect, d. This allows a more general development, independent of actual measurements, and also allows calculation of heritabilities in the various model situations. It also allows comparison of some properties of diploid and autotetraploid phenotypic distributions.

Defining σ_e in relation to d also allows the criteria for distinguishing between continuous and discontinuous phenotypic variations to be seen in the context of the genotypes. The criteria are dependent on the distribution of the environmental- or residual effects, and so far we have discussed normal distribution only. For almost any distribution, $\mu \pm 4{\cdot}\sigma$ will cover 99% or more of the observations. Consequently, if the largest distance in phenotypic value between two adjacent genotype classes exceeds $8{\cdot}\sigma_e$, the phenotypic distribution is expected to be discontinuous, irrespective of density function of environmental effects. This is, however, a very conservative border, and a normal density function is probably the best approximation in most cases.

As pointed out, the distinction between a quantitative and qualitative type of phenotypic distribution relies neither on the magnitude of the genetic effects, nor on the number of segregating loci involved. The distinction relies entirely upon the relation in magnitude between genetic effects and the standard deviation of environmental/residual effects. As can be seen from Figs. 1-6, phenotypic distribution for variation at a single locus may in many cases, even with skewed genotypic distributions and medium heritabilities, be difficult to distinguish experimentally from a normal distribution.

Another implication of the models described is that for any given degree of dominance, h/d, it is possible to calculate the minimum broad sense heritabilty, h_b^2, for a qualitative character, which is also the maximum h_b^2 for a quantitative character with the same h/d. For instance, for the diploid model and allele frequency $u=0.5$, this h_b^2 is 0.95, 0.90, and 0.87, as h/d takes the values 0, 0.5, and

1.0, respectively. If a 5% uncertainty in classification is accepted in the model, the expected borderline h_b^2 drops to 0.91, 0.83, and 0.79 for the same degrees of dominance. The example with plant height in peas (Table 3 and Fig. 8a) is considered discontinuous, provided an uncertainty of between 1% and 5% in classification is accepted in the model.

However, a large proportion of the characters one would be interested in do not constitute single locus characters. How does this simple model touch upon realities? If the loci regulating a character are independent, their effects may simply be added. Interacting pairs of loci on the diploid level are to some extent, comparable to some of the situations illustrated for the single autotetraploid locus. On the other hand, if variation in a character is dominated by either (a) one locus with a "major gene", or (b) one "effective factor", then single locus models should be sufficient to describe the phenotypic variation. The "effective factors", however, are not likely to maintain their integrity over generations, (Mather & Jinks 1971), particularly not in outbreeding species (Allard 1988).

Earliness in meadow fescue and average plant diameter in cocksfoot are certainly regulated by more than one locus, and the populations used in the experiments mentioned above, most probably segregate for more than one locus for each of the characters. Nevertheless, in both of these examples, and several unpublished ones, a single locus model with genotype frequencies as expected for populations at equilibrium, describes the phenotypic distribution adequately. Now, both simulation studies (Bulmer 1976) and studies of plant populations (Allard l.c.) clearly show that directional selection tends to create linkage blocks in the genome. These blocks will behave as (more or less) distinct, discrete genetic units, and clearly tend to produce phenotypic distributions which may be described with fewer effective factors than the number of loci involved. Both the above-mentioned characters in the populations used here have been subject to directional natural selection during adaptation to photoperiod.The considerable efforts world-wide to locate genes with large effects on quantitative traits by means of RFLPs will likely greatly contribute to a better understanding of the constitution of these «effective factors» and their contribution to phenotypic variation and distribution.

SUMMARY

Diploid and autotetraploid single locus models for phenotypic variation and distribution are described, where genotypes have panmictic equilibrium frequencies and environmental- (or residual) effects are normally distributed. Genotypes and environmental effects are assumed to be independent, and no genotype by environment interaction is assumed. The standard deviation of environmental effects is specified in relation to the additive genetic effect, *d*, as are also the non-additive effects intra loci. This allows calculations of heritabilities for any combination of parameter values in the models.

The effects on the phenotypic distribution of varying gene frequencies, degrees of dominance, and different levels of environmental variance are illustrated in a number of graphs. (Figs. 1-3 for the diploid model, Figs. 4-6 for the autotetraploid.)

The broad sense heritability in the situations illustrated varies between 0.13 and 0.94 and between 0.11 and 0.98 for the diploid and autotetraploid models, respectively. The situations span both continuous and discontinuous variation for both models. It is pointed out that the distinction between the two types of variation is reliant on the magnitude of the standard deviation of the environmental (or residual) variation relative to the largest distance between two consecutive genotypic classes. Neither the absolute

value of the genetic effects per se, nor the number of loci involved, is an efficient criterion for such a distinction. For instance, one may expect a normally distributed phenotype and a narrow sense heritability of 0.33 for variation at a single diploid locus. (Fig. 2 (2, 3)).

The single locus models have been fitted to describe the following observed phenotypic distributions:
(1) Variation for plant height and internode length among 142 F_2 plants of peas (diploid with gene frequency 0.5, Fig. 8).
(2) Variation in days to heading among 200 randomly selected clones of *Festuca pratensis* (diploid, outbreeding, unequal gene frequencies, Fig. 9).
(3) Variation in average plant diameter among 200 randomly selected clones from each of two populations of *Dactylis glomerata* (autotetraploid, outbreeding, Fig. 10).

In all cases the simple one locus model adequately describes the observed phenotypic distribution (none-significant Chi-squares for goodness-of-fit). One possible explanation for the easy fit of single locus models to characters (presumably) regulated by more than one segregating locus is the presence of linkage groups generated under directional selection. In the present cases natural selection related to adaptation to photoperiod.

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EFFECTS OF OZONE ON GROWTH AND DRY MATTER PARTITIONING IN DIFFERENT PROVENANCES OF NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.)

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Six provenances of Norway spruce seedlings were grown at different ozone concentrations over 50 or 100 days in two experiments. Ozone levels of 80-100 ppb 7-8 h day-1 reduced top and root weight as well as plant height in four of the provenances, while the other two were unaffected compared with the control treatment (10-30 ppb). No effect of ozone on top/root dry weight ratios was recorded. Ozone significantly increased the needle/stem dry weight ratios in the two provenances where this ratio was measured. Two provenances which were grown at an intermediate ozone concentration (57 ppb) showed no growth reductions compared with the control.

Key words: Growth, Norway spruce, ozone

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Up until recently little attention has been paid to ozone pollution and its effects on agricultural crops and forest trees in Norway. In many countries ambient ozone concentrations have been shown to reduce growth in different species (Adams et al. 1984; Commission of European Communities 1986, 1988). Despite a high latitude location with relatively clean air compared with most other countries (Joranger et al. 1986), the ozone concentration in Norway during summer varies between 20 and 60 ppb (Hoem et al. 1988). The high ozone concentrations (> 50 ppb) are generally related to long-range transported air masses from the European continent by southerly winds.

Norway spruce is the most important forest tree species in Norway, and therefore a study of the effects of ozone on this species is of particular interest. During recent years this tree species has also been an important one in ozone research in other countries. In the present investigations the effects of high ozone concentrations have been studied on six provenances of Norway spruce.

MATERIALS AND METHODS

Experiment 1. Two experiments were carried out. Experiment 1 comprised four provenances (B2, B3, B4, C1) of Norway spruce from Sønsterud nursery. They were sown on 1 March 1987 in multipots

(780 pots per m^2), each pot containing 50 ml substrate (Räsänen 1981). The substrate consisted of a mixture of 25% perlite and 75% peat (superfine, Hasselfors AB, Sweden). The peat was limed with 1 kg dolomitic limestone per m^3. Ninety potted plants of each provenance were placed in each of four growth chambers (Mortensen 1982) on 25 June 1987. Two chambers were supplied with ozone 8 h day^{-1} (0900-1700 h) while the other two were not. The initial top dry weight per plant varied between 0.20 and 0.23 g, the root dry weight between 0.065 and 0.088 g, and the plant height between 9.3 and 11.2 cm in the different provenances.

The plants were harvested on 5 October 1987.

Experiment 2. Two provenances (B2 and B5) of 1-year-old Norway spruce plants were potted in vermiculite (grade 1) in 8 cm pots on 9 May 1988. On 10 May 14 pots of each provenance were placed in each of six growth chambers at three different ozone concentrations 7 h day^{-1} (10.00-17.00 h), two chambers to each concentration. The initial top dry weight was 1.1 and 1.0, root dry weight 0.22 and 0.34 g, and plant height 20.2 and 18.3 cm, in B2 and B5 respectively. The plants were harvested on 5 July 1988.

During the experiments the plants were watered with a complete nutrient solution consisting of (mg l^{-1}):

N	P	K	Ca	Mg	S
158	32	209	103	35	32

Fe	Mn	Zn	Cu	B	Mo
1.8	0.8	0.16	0.11	0.16	0.03

Ozone was generated by a high-voltage generator (Nomizon, Nordmiljø AB, Sweden) using air. The high ozone concentration in Experiment 1 was adjusted to 80-100 ppb and was checked several times a week. The ozone concentration in the control treatment varied between 10 and 30 ppb because of the ambient air concentration of ozone. In Experiment 2 the ozone concentration was measured four times an hour by means of a Dasibi Environmental Corp. (Model 1008AH) analyser. Concentrations varied within +/-10 ppb at all three ozone levels in Experiment 2. The concentration of nitrogen oxides at the highest ozone treatment was 6-10 ppb, which originated partly from the ozone generator (4-5 ppb) and partly from the background air (2-5 ppb). The nitrogen oxides were measured using a Monitor Labs Inc., Model 8840 analyser. The mean temperature, air humidity, photosynthetic active radiation (PAR) and ozone levels are given in Table 1.

At termination of the experiments the dry weights of the different plant parts, plant height and number of lateral shoots were recorded. A two-way analysis of variance was carried out.

RESULTS

Ozone concentrations of 80-100 ppb significantly reduced the total dry

Table 1. The experimental period, mean ozone concentration, temperature, relative humidity and solar radiation (PAR) during the experiments

Exp. no.	Time (days)	O3 conc. (ppb)	Temperature (°C)	% RH	Radiation (mol $m^{-2}day^{-1}$)
1	105	10-30, 80-100	21.0 ± 2.0	85 ± 5	16
2	55	27, 57, 88	22.0 ± 2.0	85 ± 5	26

Table 2. Variance ratios (F) and significance levels for the different variables in Experiment 1. The residual error mean squares are given on the bottom line. Significance levels: °,P<0.10; *,P<0.05; **,P<0.01; ***,P<0.001

		Dry weights				
	Df	Top	Root	Total	Top/root	Height
Ozone (O)	1	8.63*	17.4**	12.2**	0.03	12.7**
Provenance (P)	3	7.04**	14.4**	6.04*	15.0**	11.6**
O x P	3	2.73	4.09*	3.08°	1.94	2.98°
Ms residual error	8	0.038	0.003	0.053	0.170	5.00

Table 3. The effect of ozone concentration on growth of four spruce provenances in Experiment 1. 10-30 ppb = C, and 80-100 ppb = + O_3

	Dry weights (g)									
	Top		Root		Total		Top/root		Height	
	C	+O_3	C	+O_3	C	+O_3	C	+O_3	C	+O_3
B2	1.33	1.14	0.52	0.48	1.85	1.61	2.6	2.4	23.2	20.7
B3	1.38	1.20	0.58	0.49	1.96	1.69	2.4	2.4	22.8	20.5
B4	1.10	1.06	0.52	0.53	1.63	1.59	2.1	2.0	19.4	18.9
C1	1.19	1.21	0.47	0.44	1.66	1.66	2.6	2.9	21.2	21.3
x	1.25	1.15	0.53	0.49	1.77	1.64	2.4	2.4	21.7	20.3

weight in two of the four provenances in Experiment 1 (Tables 2 and 3). Top and root weights were similarly affected, as was plant height. In Experiment 2 total dry weight of the two provenances decreased at the highest ozone concentration while no effect of the intermediate concentration was found (Tables 4 and 5). The top/root dry weight ratio was unaffected while the needle/stem ratio increased at the highest concentration. The highest ozone level also decreased plant height and number of lateral shoots.

DISCUSSION

The growth of four out of the six spruce provenances was significantly reduced by 80-100 ppb, but without any visible injury. This is in accordance with the previous findings that spruce and pine plants generally seem to be rather tolerant of ozone pollution, at least in experiments lasting only one growing season (Wilhour & Neely 1977; McLeod et al., 1986; Barnes & Davison 1988; Lucas et al. 1988). However, variation in sensitivity to ozone seems to exist between different genotypes within the same species, as previously shown with *Pinus strobus* (Nicholson & Skelly 1977; Genys & Heggestad 1978; Trimble et al. 1982) and *Pinus taeda* (Adams et al. 1988; Kress et al. 1982.)

Differences in ozone sensitivity within a species may be related to different absorption rates between genotypes (Reich 1987) and/or different resistance to the absorbed ozone which may be connected to the concentration of ascorbic acid in the cell wall (Chameides 1989)

Table 4. Variance ratios (F) and significance levels for the different variables in Experiment 2. The residual error mean squares are given on the bottom line

		Dry weights								
	Df	Needle	Stem	Root	Top	Total	Needle/ stem	Top/ root	No.side shoots	Height
Ozone (O)	2	4.21°	6.89*	4.64°	5.28*	5.51*	6.43*	0.35	9.07*	11.0**
Provenance	1	0.25	0.006	15.0**	0.08	0.95	9.29*	12.1*	30.2**	0.09
O x P	2	0.40	0.171	0.48	0.26	0.08	1.96	0.62	0.54	0.55
Ms residual error	6	0.398	0.175	0.165	1.06	1.87	0.028	6.70	12.3	61.0

Table 5. The effect of ozone concentration on growth of two provenances of Norway spruce (mean values) in Experiment 2.

Ozone conc.	Dry weights (g)								
(ppb)	Needle	Stem	Root	Top	Total	Needle/ stem	Top/ root	No.side shoots	Height (cm)
27	1.75	1.25	0.91	3.00	3.91	1.43	3.89	13.8	31.6
57	1.65	1.19	0.86	2.85	3.71	1.42	3.57	13.4	31.3
88	1.41	0.96	0.68	2.38	3.07	1.53	3.98	11.2	27.1

or other antioxidants (Mehlhorn et al. 1986).

The 7-h daytime mean concentration at Ås during Experiment 2 (50 days) in 1988 was 41 ppb. The results indicate that this ozone concentration will not reduce growth of spruce seedlings during one growing season. Plants exposed to ozone during several seasons, however, may be negatively affected by such concentrations. Sutinen et al. (1989) found that in a clone of Norway spruce ambient ozone levels (27-32 ppb, 7-h seasonal means for 1985-1987) compared to clean air (5 ppb) caused ultrastructual damage and reduced photosynthetic rates of the needles after two years. Long-term experiments are therefore needed if the effect of ozone on conifers is to be evaluated. Ozone may also decrease the frost tolerance of Norway spruce without any visible effect on growth during the growing season (Brown et al. 1987; Barnes & Davison 1988). The relationship between ozone and the hardening process in spruce seems to be the most interesting one when dealing with ozone effects on spruce. Further work should therefore be particularly concentrated on this relationship using genotypes of different susceptibility to ozone. The effect of ozone may also depend on the climatic conditions such as light, temperature, air humidity and drought (Mortensen 1989). Experiments for studying the effect of ozone in climatic conditions which maximize and/or minimize the ozone effect should therefore be carefully designed so that it is possible to make an evaluation of the potential risk of ozone damage on forests.

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EFFECT OF POT COLOUR ON THE GROWTH AND DEVELOPMENT OF POINSETTIA

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Bævre, O. A. 1990. Effect of pot colour on the growth and development of poinsettia. Norwegian Journal of Agricultural Sciences. 4: 67-70. ISSN 0801-5341.

Plants of the cv. PLA 'Anette Hegg Vinterstar' were grown in different coloured plastic pots in combination with different lengths of shading period from planting. An ordinary culture programme was used. Root growth in the white and terracotta coloured pots showed a positive gravitropic curvature, which was most pronounced in the white pots. In the black pots roots have a diageotropic growth, the geotropic stimulus in this case being related to white and red light penetrating the pot wall. Pot colour has a certain bearing on plant development. These observations are assignable to the culture method, which includes growth regulation and an adequate supply of water and nutrients. Use of black pots is preferable, because the visible roots makes it possible for the grower to detect root diseases at an early stage.

Key words: Gravitropic response, poinsettia, pot colour

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Growers using both terracotta coloured and black pots in poinsettia cultivation, have noticed differences in root development. Plants grown in black pots develop an excellent root system which twists around on the outside of the soil ball, while roots which grow in terracotta coloured pots are mainly located in the soil and have a downward growth when reaching the pot wall. This observation is also described by Hasek and Sciaroni (1975), who also found chlorotic foliages when using light-green plastic pots. In Norwegian commercial nurseries no significant differences in plant development related to pot colour have been found. However, it is of great interest to clear up any effect pot colour might have on the economic parameters of plants.

MATERIALS AND METHODS

Rooted cuttings of PLA 'Anette Hegg Vinterstar' were planted in fertilized peat in 12 cm diameter plastic pots on 20 August. Short-day condition (photoperiod of 10.5 h) was established from 14 September, and the experiment was terminated on 10 December. The plants were grown as single stems in an acrylic greenhouse using normal culture procedures: Air temperature at 20-21°C until late October, then gradually decreasing to 18°C in the last period before time of sale; fertilizing with a complete nutrition solution; chlormequat sprayed several times for growth retardation, and from the beginning of October, artificial lighting using high pressure sodium lamps

(SON-T), 10Wm^{-2} (PAR) measured at the top of the plants. The temperature was recorded hourly on a multipoint recorder.

Commercial plastic pots were used: Black pots (Strømberg), terracotta coloured pots (LOG) and white pots (OS-plastic). The different pot qualities were combined with different lengths of shading period in the establishing phase of the cuttings. Cheesecloth was used as the shading material 0, 3, 6, 9 and 12 days from planting the rooted cuttings in the pots. The experiment was carried out twice with two replicates, the first year, and three replicates the second year, with six plants per plot each times.

The number of cyathia with visible pistils and stamens at a given date was used as a measurement for earliness. In the first year, the cyathia were registered at the end of the experiment, while a weekly registration (three times) was carried out in the last two weeks of the second experiment. Lateral branching was registered as number of lateral shoots longer than 5 mm. The number of lateral flowering shoots is a subjective observation of lateral shoots with a marketable value. These registrations were made at the end of the experiments on 10 December. The bracts were measured twice, right angled. The height and the bract diameter of lateral flowering shoots used in the calculation is a total of all the lateral flowering shoots per plant. Wholesale prices in accordance with specific plant quality were used for the economic calculations in the second experiment.

The coefficients of correlation are given as Spearman rank correlations. The standard deviation of means is given in parentheses.

RESULTS

Root development

The experiment showed considerable differences in root growth. Development of the roots in the white and terracotta coloured pots was very similar, with a positive gravitropic effect toward the pot wall (Fig.1). Roots in the white pots displayed a more pronounced downward curving before reaching the pot wall than the roots in the terracotta coloured pots. On the other hand, the roots in the black pots were twisted around the soil ball, without any particular sign of georesponses. The roots in the black pots were thicker and longer than those developed in the other pots. The root mass was not measured, but on tearing the soil ball the quantity of roots seemed to be considerably greater in the black pots than in the other pots.

Figure 1. Root development of the poinsettia cv. PLA 'A.H. Vinterstar' grown in different coloured pots. From left to right: black, terracotta and white

Plant height

The average plant height was 24.1 (± 0.26) cm the first year and 24.5 (± 0.42) cm the second year. In the first year the plants grown in the black pots were significantly taller ($P < 0.05$) than the other plants. No significant differences were found the following year. The plants were significantly shorter when no shade was used ($P < 0.05$, the first year, $P < 0.01$, the second year). Regarding plant height, there was no significant interaction between pot colour and the duration of shading.

Bract diameter
The average diameter of the top bracts was found to be 18.6 (± 0.53) cm the first year and 21.2 (± 0.44) cm the second year. The plants grown in the black pots had the biggest bracts in both years, followed by those in the white and the terracotta coloured pots. These differences were significant in the first year ($P < 0.05$). Shading the plants a few days after planting, had a small but significant effect on bract diameter ($P < 0.05$). Plants which had the two longest periods of shading, produced the biggest bracts. No significant interaction on bract diameter was found between pot colour and the length of the shading period.

The diameter of the top bract did not significantly correlate with the number of lateral shoots or number of lateral flowering shoots.

Lateral branching
For the subsequent two years, 2.19 (± 0.22) and 1.39 (± 0.25) lateral shoots per plant were registered. The different types of pots had no significant effect on lateral branching in the second year. However, the effect of shading was significant ($P < 0.05$), when the longest shading period resulted in the fewest lateral shoots (0.88 per plant). No significant interaction was found between pot type and the length of the shading period.

Lateral flowering shoots
The mean number of lateral flowering shoots was 1.13 (± 0.18) the first year and 0.29 (± 0.09) the second year, without any significant effect of pot type. The highest number of lateral flowering shoots was produced without shading in both years, but the effect of the shading programme was significant only in the second year ($P < 0.05$). The number of lateral flowering shoots was positively correlated with the lateral branching both years($r = 0.53$ and $r = 0.70$, $P < 0.001$).

The total height or the total diameter of lateral flowering shoots per plant showed no significant variation with colour of the pots or with the length of the shading periods. Concerning the number of lateral flowering shoots, no significant interaction was found between pot colour and the length of the shading periods.

Earliness
Pots of different colour had no significant effect on the development of cyathia. In the second year, the number of cyathia per plant increased from 0.28 (± 0.07) two weeks before sale, to 0.55 (± 0.11) one week later and 1.38 (± 0.19) at time of sale. The number of flowers at any given moment was negatively, but not significantly correlated with the number of lateral shoots or number of lateral flowering shoots.

Plant price
Classification of the plants in the first year, gave an average price of NOK 20.60 (± 0.71), which did not vary with experimental conditions.

The plant price was positively correlated with the number of lateral shoots ($r = 0.37$, $P < 0.001$), number of lateral flowering shoots ($r = 0.66$, $P < 0.001$) and the diameter of the top bracts ($r = 0.60$, $P < 0.001$).

DISCUSSION

The different chemical contents of the pots related to pot colour had no effect on root development. A possible chemical effect was tested in a previous experiment (not published) when two pots of different colour were used inside each other (Fig. 2). The observed differences in geotropic responses in plants grown in the differently coloured pots must be related to light penetration through the pot wall. Light is an essensial factor in a positive gravitropic response for many plants. Lake & Slack (1961), Scott & Wilkins (1969), Suzuki & Fujii (1978)

Figure 2. Root development of the poinsettia cv. PLA 'A.H. Vinterstar' grown in different coloured pots. From left to right, a tall black pot, a tall terracotta coloured pot, a tall white pot, a short terracotta coloured pot, a short black pot in a terracotta coloured pot and finally a small terracotta coloured pot in a black pot

and Feldman & Briggs (1987) have concluded that phytochrome is the only pigment that is involved in light-induced gravitropic bending in roots. The diageotropic growth of the roots in the black pots is related to the fact that the colour of the pots does not allow light penetration. On the other hand, the positive geocurvation observed in the other pots may be connected with light penetrating the pot wall (Suzuki & Fujii 1978, Hart & MacDonald 1980, Miyazaki et al. 1986). The poinsettia cultivar PLA 'A. H. Vinterstar' must be a plant with a light-promoted gravisensitivity in the roots. The inhibition of root growth as recognized is described for other plants, too (Ohno & Fujiwara 1967, Schwarz & Schneider 1985).

Only small differences in plant development could be related to pot colour. An increased soil temperature of 0 - 1.5°C in the black pots in comparison with the other pots at the beginning of the season, especially in sunny weather, is not expected to give responses on plant growth at the fixed temperature regime (Janes & McAvoy 1982).

The decreasing daylight intensity in the autumn, a normal culture procedure including growth regulation, a complete nutrient solution and a flow watering system contributed toward neutralizing any possible effect of pot quality. It is likely that plants grown under these conditions will receive nutrition and water regardless of root distribution. On the other hand, black pots, which give visible roots, are preferable. Visible roots allow the growers better to inspect the roots for possible diseases.

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THE EFFECT OF CO_2 ENRICHMENT ON TOMATO YIELD DURING GREENHOUSE VENTILATION

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The effect of CO_2 enrichment during periods of ventilation was studied in eight tomato greenhouses in 1985 and 1986. Four of the greenhouses received 2.75 kg CO_2 1000 m^{-2} h^{-1} in periods of ventilation while the other four did not. This enrichment resulted in CO_2 concentrations of 300 to 450 $\mu l\,l^{-1}$, depending on the vent opening. In houses without enrichment the concentration was from 260 to 300 $\mu l\,l^{-1}$ during ventilation. In periods when the vents were closed all greenhouses were maintained at a concentration of 900 $\mu l\,l^{-1}$.
The average yield for 1985 and 1986 in the greenhouses with enrichment during ventilation periods was 32.9 kg m^{-2}; without enrichment the yield was 33.0 kg m^{-2}. The total CO_2 consumption during ventilation periods was 6.1 kg m^{-2}. It is concluded that CO_2 enrichment during ventilation periods with the present CO_2 flow rate does not affect the yield.

Key words: CO_2, greenhouse, tomato, ventilation.

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In greenhouses without ventilation and without CO_2 application, the CO_2 concentration could fall to below 200 $\mu l\,l^{-1}$ (Sebesta & Reiersen 1981, Slack & Hand 1985, Slack et al. 1988). Even with ventilation the concentration could decrease significantly below the outside concentration. Levels of 200-300 $\mu l\,l^{-1}$ have been reported (Drakes 1984, Slack & Hand 1985). In England, carbon dioxide enrichment during periods of ventilation has been found to give positive effects on tomato yield (Drakes 1984, Slack et al. 1988); this is also to some extent the case in Denmark (McCall & Saxe 1987). In the English and Danish studies a constant CO_2 concentration was maintained irrespective of the vent opening. In the present study the CO_2 concentration was kept at 900 $\mu l\,l^{-1}$ when the vents were closed, while a constant CO_2 flow was supplied when the vents were open.

MATERIALS AND METHODS

Eight greenhouses were chosen in three greenhouse holdings in south-west Norway (59°N) (Table 1). Most tomato greenhouses in Norway are located in this area. Summer temperatures of 10-20°C a rainfall of 2-4 mm day^{-1}, relative air humidities (RH) of 75 to 85%, a photon flux of 15-30 mol $m^{-2}day^{-1}$ (PAR) and south westerly winds are typical of

Table 1. Greenhouse sizes and tomato cultivars (Virosa = V and Abunda = A) in the different experiments in 1985 and 1986

Greenhouse pair	Greenhouse size (m^{-2}) Control	+ CO_2	Cultivar 1985	1986
A	1040	960	A	A
B	1100	900	V	A
C	560	560	V	V
D	915	680	V + A	-

Table 2. Photon flux (PAR), mean daily maximum and minimum temperatures, relative humidity (RH) and mean daily rainfall during the experimental period (Meteorological data, Særheim Experimental Station, 1985, 1986)

Period	PAR (mol m^{-2} day^{-1}) 1985	1986	Temperature (°C) 1985 max	1985 min	1986 max	1986 min	% RH 1985	1986	Rainfall (mm/day) 1985	1986
5.2-15.4	9.7	14.7	6.3	3.2	6.0	2.2	76.2	76.0	1.7	1.7
15.4-1.10	26.0	28.2	16.6	10.3	16.7	12.8	82.6	86.1	3.4	3.6

this area (Table 2). All the greenhouses were given 900 µll^{-1} CO_2 from the start of the experiment in the middle of February until termination (1 October) for as long as the vents were closed. Half of the greenhouses were supplied with 2.5-3.0 kg CO_2 1000 m^{-2} h^{-1} (mean 2.75 kg) when the vents were open. The other half were not given CO_2 during the ventilation periods. The CO_2 gas was supplied from bottles through perforated tubes which were placed between each double row on the greenhouse floor. Two flow controllers were used, one for application of the CO_2 when the vents were closed, in order to maintain 900 µll^{-1} CO_2 (about 4.0 kg CO_2 1000 m^{-2} h^{-1}), the other for application of 2.75 kg CO_2 1000 m^{-2} h^{-1} when the vents were open in half of the greenhouses. The gas was supplied in the light period only.

When planning the experiments care was taken to choose pairs of greenhouses which in earlier years had given approximately the same yield (within a 0.0-1.0 kg m^{-2} difference). One of the greenhouses in each pair was given CO_2 during the ventilation periods, but not the other. Four pairs of greenhouses (A,B,C,D) were included in the experiment in 1985 and three in 1986. Greenhouse pairs and tomato cultivars are shown in Table 1. Pair A was located in one greenhouse holding, pair B in a second, and pairs C and D in a third. Outside climatic conditions during the experimental period are given in Table 2.

The tomato plants were raised under artificial light and planted out in the greenhouses (2.5 plants m^{-2}) in rockwool (Grodan) between 10 and 20 February. Night temperature until 15 April was maintained at 18-20°C, thereafter at 16-18°C. The ventilation temperature until 15 April was 25-26°C, and thereafter at 22-24°C. The RH level was kept at 80-85%, except in the pollination period between 10 and 12 a.m. when the humidity was lowered to 75-80% by means of heating and ventilation. In this period trusses with flowers were agitated using a battery-driven vibrator.

The commercial greenhouses included in the experiments had no continuous measurements of the CO_2 concentration. The concentration was therefore measured continuously in one of the greenhouses (greenhouse pair C, + CO_2) at different vent openings at different CO_2 flow rates throughout two weeks in varying weather conditions (Table 1). The greenhouse used was 50 m long (560 m^{-2}) and was fitted with continuous ridge vents, 1 m in width. The measurements were carried out in the middle of the greenhouse 2 m above the ground by means of an infrared gas analyser (Beckman, Model 864).

Table 3. The effect of CO_2 enrichment on tomato yield during ventilation

CO_2 treatment	Yield (kg m^{-2}) 1985	1986	Mean
Control	34.4	31.4	33.0
With CO2	33.4	32.3	32.9
No. of replicates	4	3	7
Significance at P = 0.05 level	ns	ns	ns

RESULTS

Enrichment with CO_2 during periods of ventilation did not significantly affect the tomato yield in 1985 or 1986 (Table 3). The mean numbers of hours with CO_2 enrichment were 2210 and 830 with the vents open and closed, respectively (Table 4). From the start of the experiment until 15 April CO_2 enrichment was supplied for approximately 50 h with the vents open and for 450 h with the vents closed. The total CO_2 consumption during the experiment were 6.1 and 3.3 kg CO_2 1000 m^{-2} h^{-1} with vents open and closed, respectively.

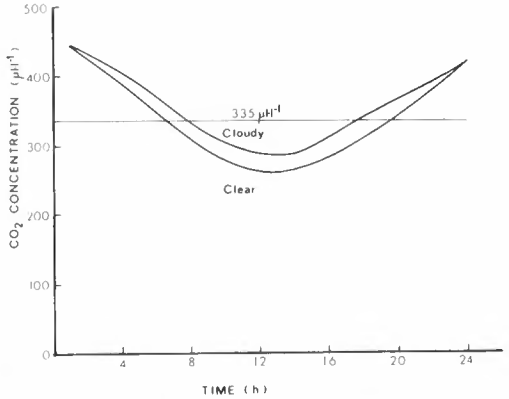

Fig. 1.T he diurnal CO_2 concentration in a tomato greenhouse without CO_2 enrichment in cloudy (<15 $molm^{-2}day^{-1}$ PAR) and clear weather (>40 $molm^{-2}day^{-1}$ PAR). Time 0 is midnight. Measured in mid-May.

The diurnal CO_2 concentration without CO_2 enrichment of the greenhouse air was measured throughout a two-week period in May. Typical CO_2-curves are given in Fig. 1. In cloudy weather (<15 mol m^{-2} day^{-1} PAR), with the ridge vents

Table 4. Number of hours with CO_2 enrichment with vents closed or open, and CO_2 consumption per m^2, as means for 1985 and 1986. Standard errors are given

Vents	No. of hours	CO_2 consumption (kg m^{-2})
Closed	830 ± 30	3.3 ± 0.1
Open	2210 ± 130	6.1 ± 0.3
Total	3040	9.4

open just enough to prevent an increase in humidity, the CO_2 concentration fell to 285 µll^{-1}. During sunny days (>40 mol m^{-2} day^{-1} PAR) when the vents were wide open in order to prevent temperature increases, the CO_2 concentration decreased to 260 µll^{-1}.

The CO_2 concentration was also measured when the greenhouse air was supplied with two application rates of CO_2 at different openings of the vents (Fig. 2). The photon flux density was about 800 µmol m^{-2} s^{-1} outdoors and 480 µmol m^{-2} s^{-1} at the top of the plants during the measurements. With 3.5 kg CO_2 1000 m^{-2}h^{-1} supplied the CO_2 concentration fell from 490 to 320 µll^{-1} when the vent opening increased from 2 to 34 cm (full opening). With 7.0 kg 1000 m^{-2} h^{-1} supplied the CO_2 concentrations were 1000 and 360 µll^{-1} respectively at the two vent openings.

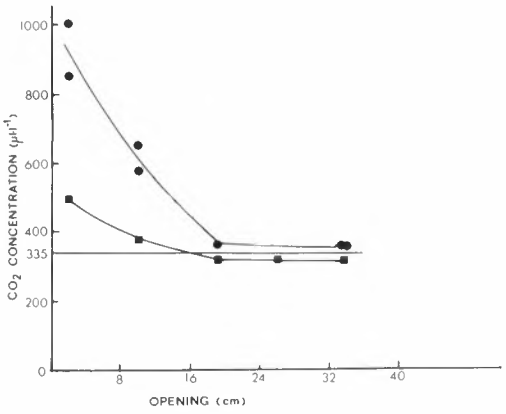

Fig. 2. The effect of the opening of the ridge vents on the CO_2 concentration in a 560 m^2 tomato greenhouse supplied with 3.5 (■) and 7.0 kg $CO_2$1000 m^{-2}h^{-1} (●). The opening of the ridge vents was measured from the outer tip of the vents down to the greenhouse roof. The outside CO_2 concentration of 340 µll^{-1} is indicated by a line

With 2.75 kg CO_2 1000 m^{-2} h^{-1} supplied during the ventilation periods, as was used in the experiment, the concentration varied from 300 (sunshine, vents wide open) to 450 µll^{-1} (clouded, 2 cm vent opening), depending on the solar radiation (data not presented).

DISCUSSION

Despite an increase in the CO_2 concentration by CO_2 enrichment during periods of ventilation the tomato yield was not significantly affected. An English experiment with tomato, where the CO_2 concentration was prevented from falling below 335 µll^{-1} during the summer, showed an yield increase of 14.5% compared to the ambient concentration (Drakes 1984). This positive effect of CO_2 enrichment, however, could be explained by the very low concentrations which were likely to occur with the vents closed in the ambient treatment. In another English study yield increases of about 5, 10 and 20 % were found by 375, 450 and 525 µll^{-1} CO_2, respectively, compared to the ambient concentration (Slack et al. 1988). These concentrations were kept irrespective of vent opening. In this report it was concluded that with between 320 and 526 µll^{-1} CO_2 the tomato yield increased by 2.65 kg for each 100 µll^{-1} increase in mean CO_2 level.

In a Danish study with tomato the effect of 1000/400 µll^{-1} CO_2 when the vents were closed/open was compared with no enrichment at all throughout the summer (McCall & Saxe 1987). The yield increased by 8 %. This effect could at least be partly explained by very low CO_2 concentration in the control treatments when the vents were closed.

Different experimental designs could to some extent explain the different results obtained by CO_2 enrichment during ventilation. However, it was expected that the CO_2 enrichment would increase the yield since it is well known that any increase in CO_2 level within certain limits should have a positive effect on the tomato yield (Mortensen 1987). When this was not the case it could partly be explained by the high

CO_2 concentration which was also applied to the control houses in periods when the vents were closed. It has been shown that the effect of CO_2 enrichment decreases with time in plants which have been grown continuously at high CO_2 levels (Aoki & Yabuki 1977, Ehret & Jolliffe 1985, Imai 1978, Mortensen 1983, Peet 1986). However, reducing the daily enrichment period has been shown to decrease the tomato yield (Calvert & Slack 1976). Another important point is that the 2.75 kg 1000 $m^{-2}h^{-1}$ application rate only increased the CO_2 concentration from 260 to 300 $\mu l l^{-1}$ at the highest PAR levels (sunny days), and from 285 to 450 $\mu l l^{-1}$ at low PAR levels (cloudy days). This means that the increase in CO_2 concentration was relatively small when the plant growth was supposed to be at its highest. It seems that a higher CO_2 concentration has to be maintained during periods of high PAR levels if an enhancement of the yield is to be accomplished. By supplying 7.0 kg 1000 $m^{-2}h^{-1}$ the CO_2 concentration could have been raised to 360 $\mu l l^{-1}$ during periods with high radiation and maximum ventilation. However, this would have substantially increased the CO_2 gas consumption.

ACKNOWLEDGEMENTS

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VI. PLANT GROWTH AND CHEMICAL ANALYSIS

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The physiological root death of cucumber was studied in plants grown individually in containers. During 2 months growth, water and nutrient uptake, nutrient distribution and assimilate production and distribution were observed. The shoot/root ratio (fresh weight) increased until harvesting; the generative/vegetative ratio increased from flowering through the whole period. The rate of fruit production and the leaf area observed indicated that assimilate production was barely enough for fruit production; other plant parts would therefore starve.
The uptake and transport of nutrients was shown to be reduced after harvesting had started. Reduction in uptake may have been caused by reduction in root surface area after root death. With the onset of fruit production it was seen that the content of non-structural carbohydrates in other plant parts had decreased. It seemed that root death in this experiment was caused by the sink strength of the fruit with regard to both assimilates and mineral nutrients.

Key words: cucumber, *Cucumis sativus*, mineral uptake, mineral distribution, root death, stachyose, starch, sucrose

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Physiological root death of the cucumber (*Cucumis sativus* L.) may be caused by competition between roots and fruits. The dominance of the fruit would affect the whole plant. Van der Post (1968) showed that shoot growth stopped and the root mass became diminished after fruit growth was initiated. Therefore the top/root ratio increased tremendously. The decrease in root mass was associated with root death (Van der Vlugt, part 4, in prep.), the order of events is not known, however.

The uptake and transport of nutrients may also be affected by fruit growth and root death. Iron deficiency symptoms have often been observed in plants with root death. Deficiencies of other elements not relocated within the plants may also be expected. Khudheir & Newton (1980) studied nutrient uptake in tomatoes in connection with root death. They found that uptake of all elements decreased in plants affected with root death and that uptake of boron and manganese decreased earlier than uptake of the other elements.

Assimilation and distribution of stachyose and other sugars in the cucumber plant have not been studied in connection with root death.

The purpose of this experiment was to study growth, mineral nutrition and assimilate production and transport in the cucumber in relation to root death. The elements investigated were N, P, K as major nutrients, Fe and Ca as elements not easily redistributed in the

plant and B and Mn as possible indicators of root death. With regard to carbohydrates, the primary product (hexoses, sucrose), the transport unit (stachyose) and the storage unit (starch) were studied.

MATERIALS AND METHODS

Seeds of the cucumber cultivar Farbio were sown in rockwool blocks (7.5 x 7.5 x 7.5 cm) in September 1985. On October 14 (= day 24), the plants were placed in 10 l containers. The rockwool block was fitted into the lid, with 1 cm protruding.

During propagation and after planting the plants received additional artificial light, 180 W/m^2 installed SON and HPI lamps, for 18 hours/day. The plants were trained upwards to the wire, and after the wire was reached the tops grew downwards. All side-shoots were pinched and all fruits were allowed to develop.

The containers were filled with nutrient solution and air was bubbled through the solution continuously. The solution was supplied from a 250 l tank, from which a sample was taken for chemical analysis, whenever the tank was refilled. The standard solution contained in ppm: 200 nitrate-N, 30 P, 420 K, 200 Ca, 1.5 Fe (chelated), 1.0 Mn, 0.5 B and adequate amounts of the other elements. The solution in the tank showed small deviations from the standard solution. The containers were refilled once every day, and the volume supplied was measured for each plant separately.

Ca, Fe and Mn in the nutrient solution were determined with a flame atomic absorption spectrophotometer. Potassium was determined with a flame photometer. The molybdo-vanadate complex with phosphorus was determined spectrophotometrically. Boron was determined spectrophotometrically with carminic acid (Rosenfeld & Selmer-Olsen, 1979). Nitrate-N was determined colorimetrically with a Technicon Auto-analyzer after reduction of the nitrate with a Cd-reductor and the addition of sulphanilamide and N-1-naphtylethylenediamine (Henriksen & Selmer-Olsen, 1970).

At planting each plant was given a number. The numbers were randomized throughout the greenhouse. Twice weekly (days 24, 27, 31, 34 etc.) 3 plants in numerical order were taken for measurements and chemical analysis. Leaves, which were not dried out and longer than 5 cm, were measured. Leaf area was calculated as length x width x 0.72 according to Graf-Marin (Liebig, 1978, pp. 17-18).

Fresh weight was determined for all plants: roots (without the rockwool block), stem segment including leaf nos. 4 and 5, top (6 uppermost unfolded leaves + stem and apex), fruit and complete shoot. Whenever the side-shoots were pinched, old leaves removed or fruits picked before the whole plant was taken, these parts were also weighed. The weight was later added to the total.

For organic chemical analysis plant parts from two plants were thoroughly mixed and extracted with 86% ethanol. The residue after filtration was used for starch determination after a modification of Pharr & Sox (1984). The filtrate was used for total sugar and stachyose determination. Total sugar was measured as glucose after a modification of the method of Hagedorn & Jensen. For stachyose determination the filtrate was concentrated and analysed by paper chromatography. Whatman no. 4 filterpaper was used, perpendicular to fibre direction, descending elution with n-butanol : acetic acid : water = 200 : 50 : 250 upper phase. Stachyose was hydrolyzed by a method developed by Mrs. G. Remedios (not published) and the hexoses were developed with silver nitrate.

The third plant was used for inorganic chemical analysis. Nitrate-N was extracted by boiling the fresh material in 0.01 M $CuSO_4$-solution; a quantitative determination was made

with an ion-selective electrode (Øien & Selmer-Olsen, 1969). The other elements were determined in a hydrochloric acid solution (5%) of the ash. Ca, Fe and Mn were determined with flame atomic absorption spectroscopy. P was determined colorimetrically as the molybdovanadate complex (Boltz et al., 1977). Potassium was determined in a flame photometer. Boron was determined with a spectrophotometer as a complex with carminic acid in concentrated H_2SO_4 (Oelschlager, 1956).

Total weight and total leaf area per plant were calculated for all days of observation. Curves were fitted using a spline routine that minimized a linear combination of the sum of squares of the residuals of fit and the integral of the square of the second derivative (Reinsch, 1967).

RESULTS AND DISCUSSION

The plants developed very well. However, from the end of November (day 66) excessive ageing of the plants occurred. Flowering occurred on day 38, the first fruits were harvested on day 55 and root death occurred on day 62.

In another experiment (not published), the average volume of the roots was observed to be about 0.2 l. This did not seriously affect the calculation of the uptake of nutrients until the end of the experiment when a more diluted topping-up solution was used.

Root weight was more or less constant after a short while (Fig. 1A), but shoot weight continued to increase (Fig. 1B). The shoot/root ratio increased. Leaves 4+5 were followed throughout their development, their weight showing a slight decline towards the end as is usual (Ho et al., 1984). Also, the top of the plant decreased in weight after harvesting had started. Pharr & Sox (1984) noticed that all plant parts above the fruit decreased in weight.

The weight of the total fruit mass increased almost linearly with 96 g fresh weight per day (Fig. 1C). Fruits were harvested when they weighed 300-400 g. Between two successive harvests the fruit mass increased by 300-400 g. Several fruits developed at the same time, which meant that the plant carried a load of at least 500 g fruit from day 55 onward.

On average, the dry matter content of the fruits in this experiment was 3.6%. The increase in dry matter was 3.5 g CH_2O assimilated per day, which concurred with the results of Pharr et al. (1985). Total leaf area reached a maximum of about 150 dm^2 on day 52 (Fig. 2). Afterwards the total leaf area decreased because of excessive ageing. For the production of 3.5 g CH_2O 5 g CO_2 is needed. The average leaf used 170-210 mg $CO_2/dm^2/day$ in the experiment of Schapendonk & Challa (1980), who used a 14-hour photoperiod. The average leaf area was 5.5 dm^2 in this experiment. For 3.5 g CH_2O one would need 5-6 leaves giving a total area of 27.5-33 dm^2.

The average water uptake varied considerably (Fig. 3). Up until root death, variation in water consumption concurred with variation in radiation (Anon., 1986). After root death, the correlation was less clear.

The EC in the containers increased gradually; therefore, at the end of the experiment a more diluted topping-up solution was used. Because of the calculation method, uptake of nutrients was in time with water uptake, only at the end of the experiment did nutrient uptake decrease. The EC was not taken into account in calculating nutrient uptake, so this may have led to overestimation of the actual amount taken up. Still, our results more or less concur with the literature, e.g., the average water consumption in this experiment was 0.9 l per plant per day. This estimate was low because there were more plants at the beginning of the experiment. A maximum of 467 mg nitrate-N and 651 mg K

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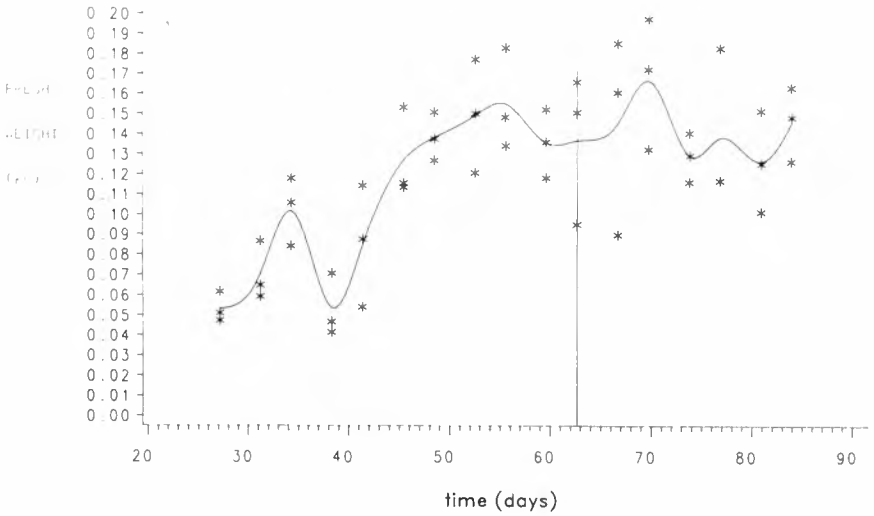

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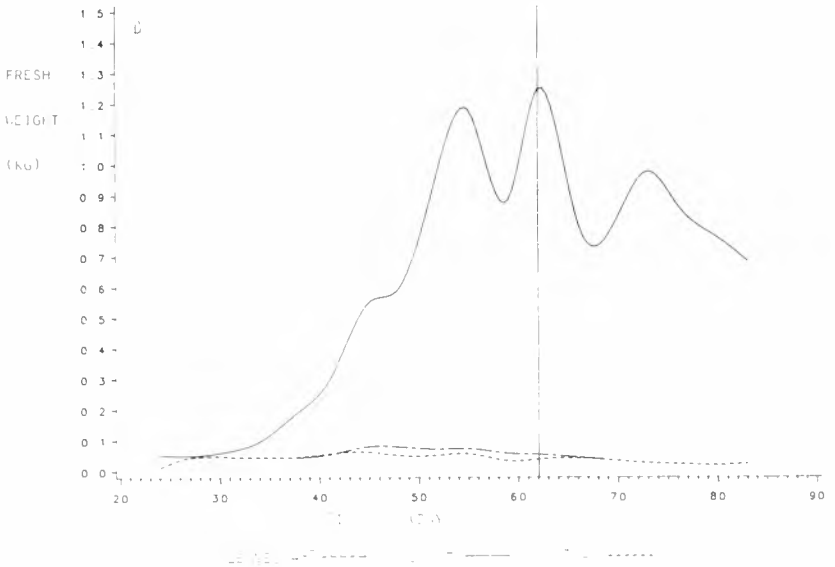


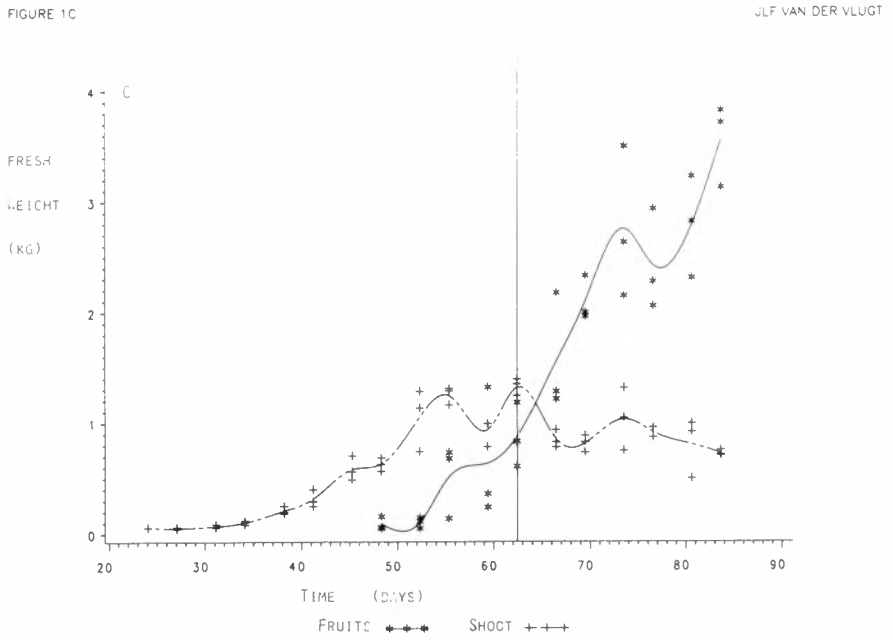


Fig. 1. Development of fresh weight (kg) in different plant parts of the cucumber. Root death occurred on day 62. A. Root weight per plant. B. Weight distribution in the shoot per plant. C. Weight of the whole shoot and all fruits per plant

were taken up according to the calculation with 2.4 l water. Adams (1980) found in his plants an average uptake of 1.14 l water with 250 mg nitrate-N and 380 mg K per day. Even higher rates have been found in the tomato (Adams & Massey, 1984).

Distribution of the nutrients in the different plant parts is shown in Fig. 4. The nitrate content in the roots was not affected by root death (Fig. 4A). The nitrate content of leaves 4+5 showed a peak on day 48. Nitrate is reduced in the leaves (Matsumoto & Tamura, 1981). Both fruit and shoot contained a considerable amount of nitrate on the day of root death. The total content of nitrate-N remained constant after root death, though this was differently distributed between shoot and fruit; probably the shoot lacked sufficient energy to reduce the nitrate.

The amount of phosphorus in the roots increased gradually after a minimum on day 41 (Fig. 4B), The amount of P in the fruit increased while it decreased in the shoot; the fruit seemed to acquire P at the expense of the shoot, while transport from the roots seemed to be failing.

The leaves, and to a lesser degree the tops, contained most of the potassium in the shoot (Fig. 4C). This is noticeable when the plants are young. The potassium content in the roots remained constant after root death, while it decreased

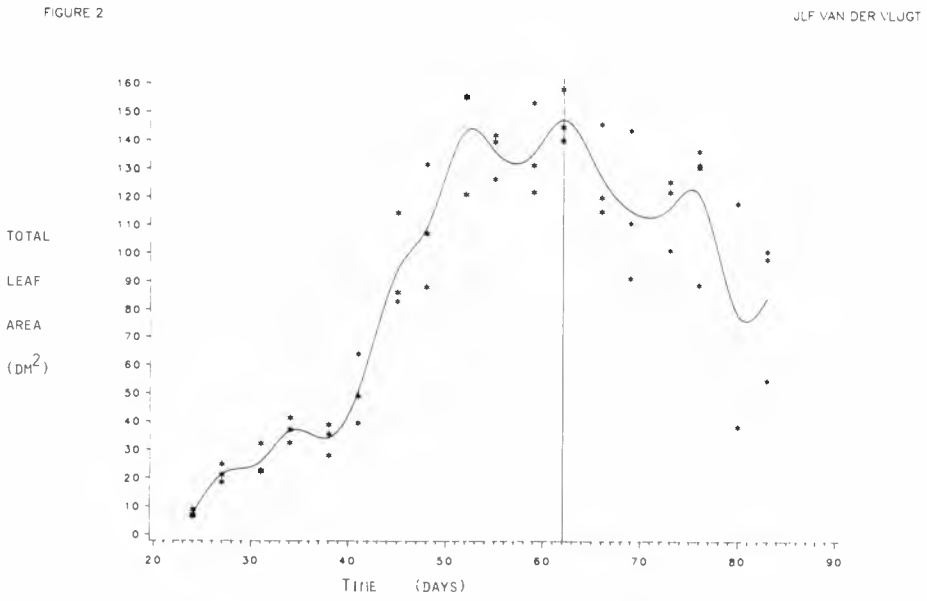


Fig. 2. Total leaf area (dm^2) per plant. Cucumber leaves were longer than 5 cm and not dried out. Root death occurred on day 62.

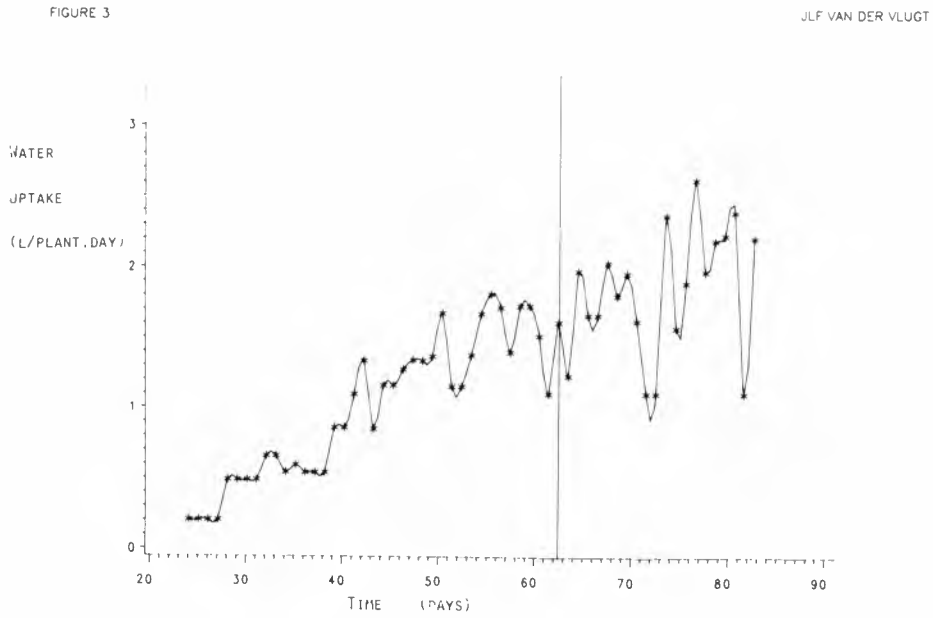


Fig. 3. Average water uptake (l/plant per day) of the cucumber. Root death occurred on day 62

in the shoot. The uptake by the fruit continued, seemingly at the expense of the shoot.

The fruit contained very little calcium (Fig. 4D). A low calcium content was found in the shoot around the time of root death. Calcium uptake and transport seemed to be little affected by root death. Root death occurred at some distance from the root tip. Since calcium is taken up by the distal 20 cm of the Cucurbita root (Clarkson 1981), uptake might not have been affected so much.

The iron content of the root was high and increased more rapidly after root death (Fig. 4E). The increase in shoot and fruit was less. Wanasuria & Kuhn (1977) observed that transport of iron may have failed because of precipitation of iron phosphate in the roots.

Although the boron content of shoot and fruit increased steadily, it decreased per fruit (Fig. 4F). The manganese content increased in the root (Fig. 4G) and it reached a maximum in the shoot on day 55. Thereafter the fruit may have obtained manganese through relocation rather than through transport from the roots.

Ward (1967) analysed leaf blades, petioles, stem, roots and fruits separately for N, P, K and Ca. Only leaves 4+5 and roots were compared with his results. In this experiment we found a higher content for all elements in these plant parts, except for N in the leaves and Ca in the roots, which concurred with Ward's results. On the other hand, the content of minerals never reached toxic levels, as given for boron by Bergmann et al. (1965) and for manganese by Osawa & Ikeda (1974) and Geissler et al. (1976).

Summing up, it would seem that mineral uptake and transport were both affected by root death, but differently for the different elements. The reduced uptake might be explained by the reduction in root surface area. Van der Vlugt (part 4 in prep.) showed that the stele diameter is only one-third of the root diameter. In root death, the cortex up until the endodermis is destroyed (ibid.) and the mere reduction in surface area could explain a reduction in uptake by two-thirds. Not the whole root system is affected simultaneously, but debris from the cortex may make diffusion more difficult. A reduction in uptake of 0-60% could be expected. Transport of nutrients may have been diminished because of lack of assimilates in the roots, which is supposed to be the case in root death.

Fig. 5 shows the content of sucrose, stachyose and starch 3 hours after the lights were switched on. Stachyose was used immediately in the roots (Fig. 5A). The total content of non-structural carbohydrates was low from days 45 to 73, after which time especially the level of starch increased. The starch content of leaves 4+5 decreased with age, while the sucrose content varied. The stachyose level was low all the time (Fig. 5C).

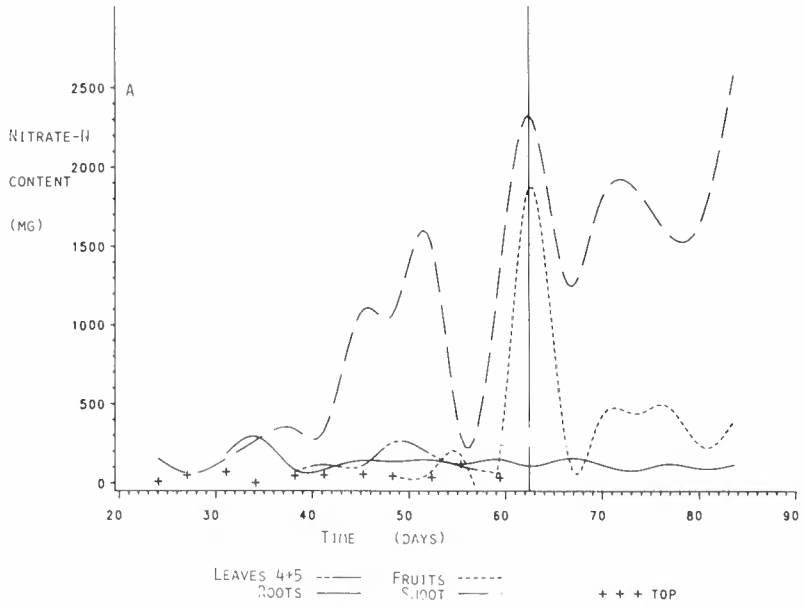
In the top, the content of all carbohydrates analysed decreased after flowering (Fig. 5B). Possibly the starch level increased again after day 73, which would be in contrast to the decrease in weight observed. The fruit mainly contained sucrose, the content of which for individual fruits decreased towards the end of the experiment (Fig. 5D).

The results indicated that the fruit is the strongest sink for assimilates also. It can monopolise up to 95% of the assimilates from the nearest leaves (Murakami et al., 1982), or 80% of the total production of the plant (Barrett & Amling, 1978).

CONCLUSION

It would seem that in this experiment competition for assimilates was the main factor triggering root death. Assimilate production was barely sufficient for fruit growth. Because of starvation, root-bark cells died, which diminished the area through which mineral uptake could take place. Also, the dead cells may have hindered diffusion to the living part of

FIGURE 4A JLF VAN DER VLUGT



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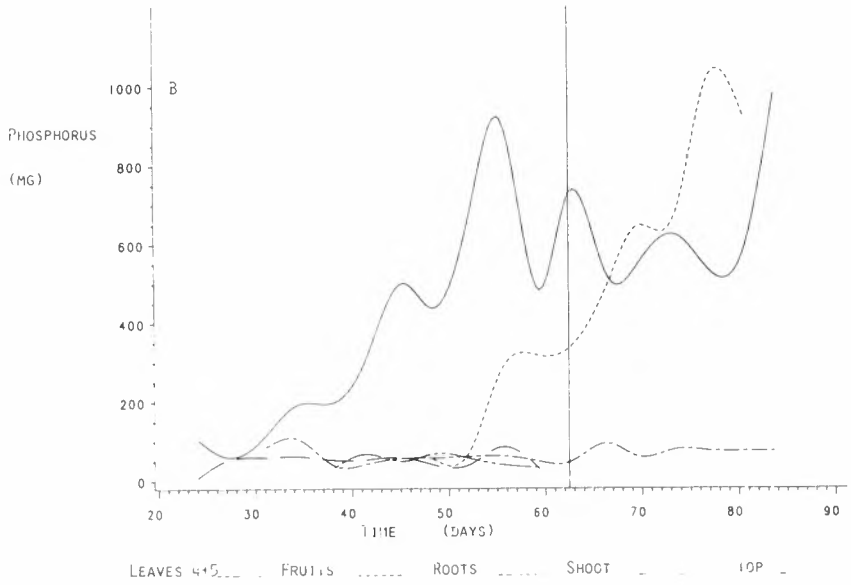

FIGURE 4C

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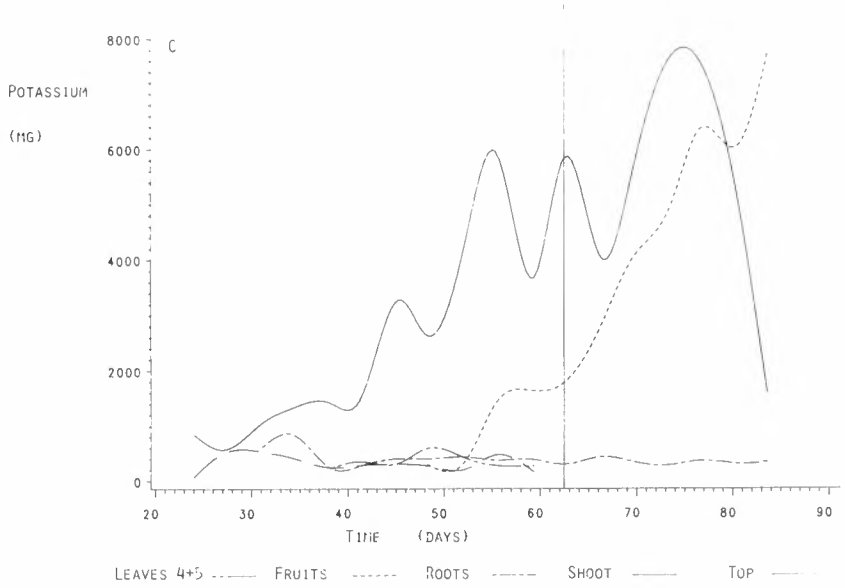

FIGURE 4D

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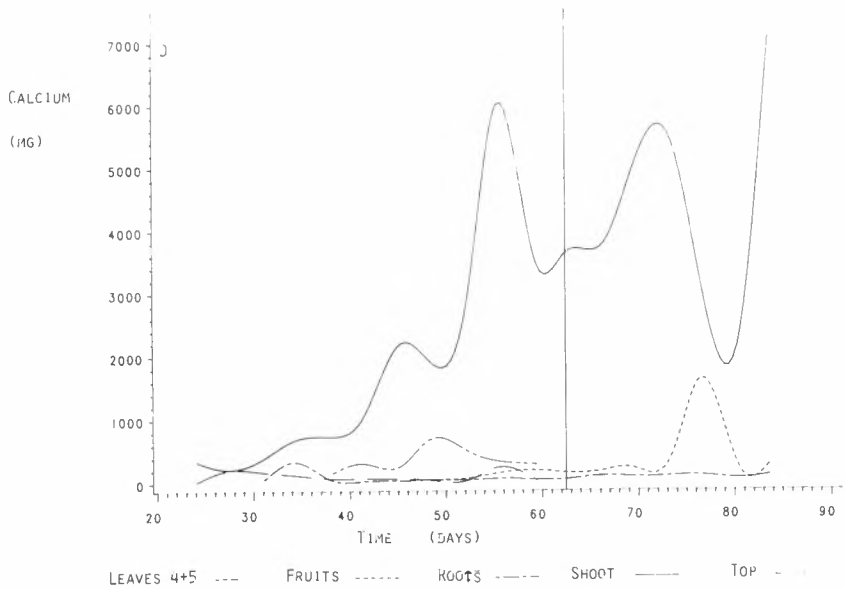

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FIGURE 4E

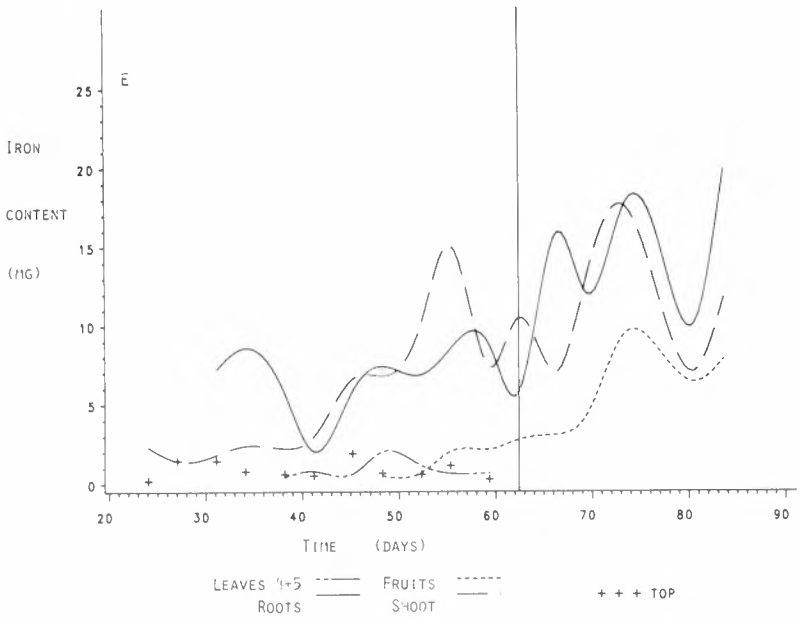
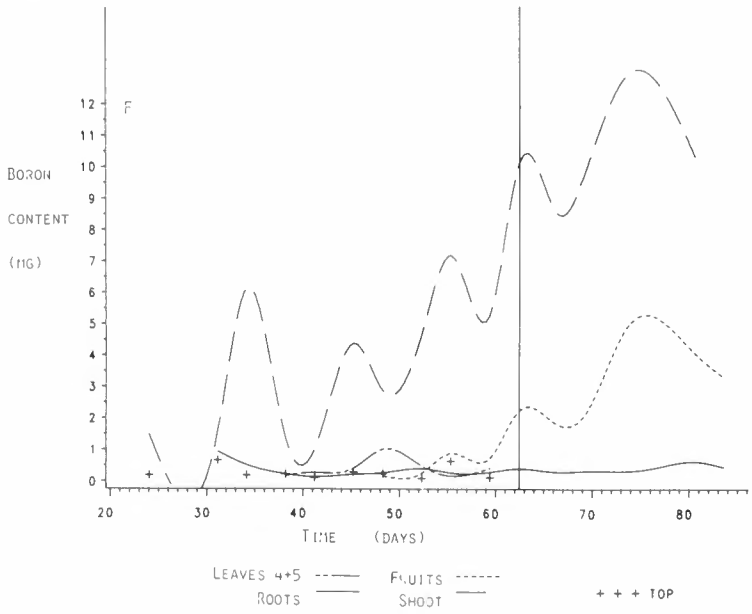


FIGURE 4F

JLF VAN DER VLUGT



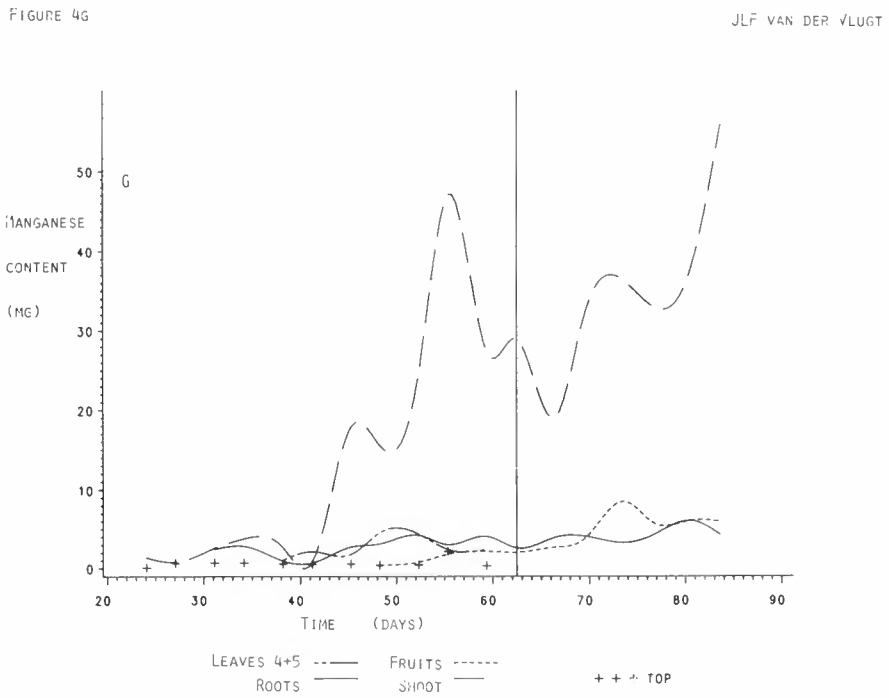


Fig. 4. Actual content (mg) of different minerals in different parts of cucumber plants 24-83 days old. Root death occurred on day 62. A. Nitrate-N content B. Phosphorus content C. Potassium content D. Calcium content E. Iron content F.Boron content G. Manganese content.

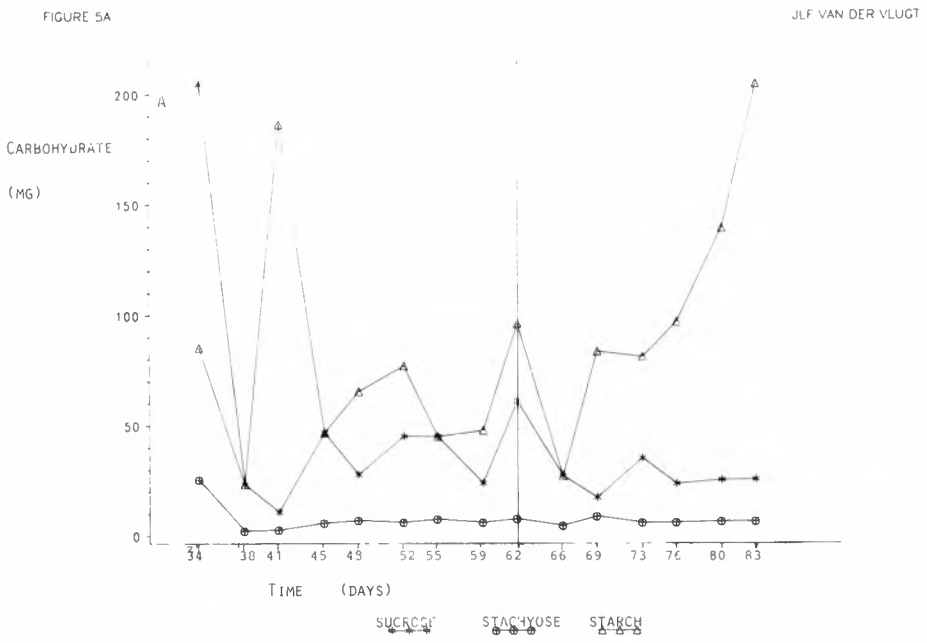


FIGURE 5B JLF VAN DER VLUGT

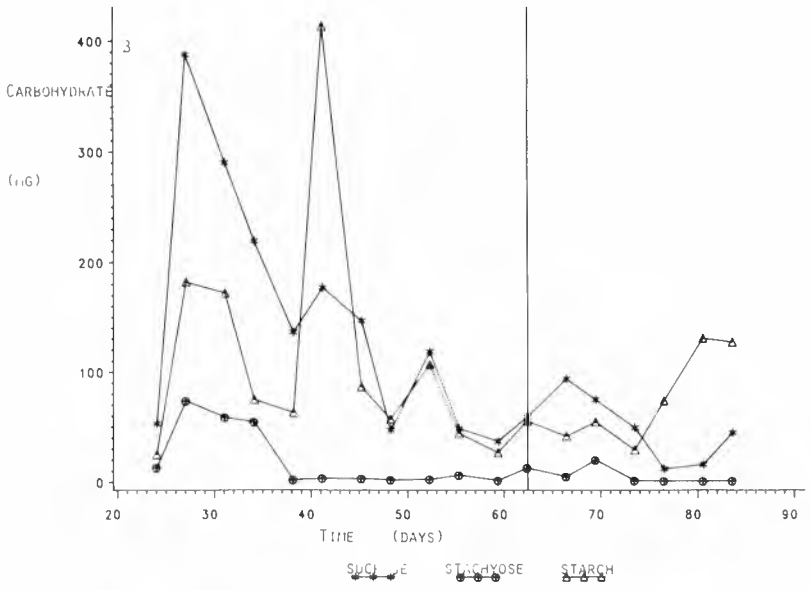
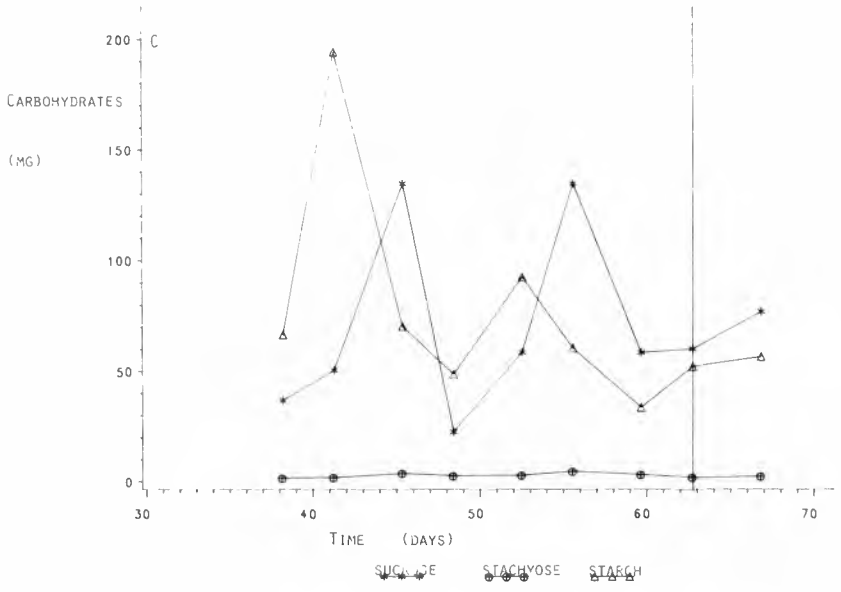


FIGURE 5C JLF VAN DER VLUGT



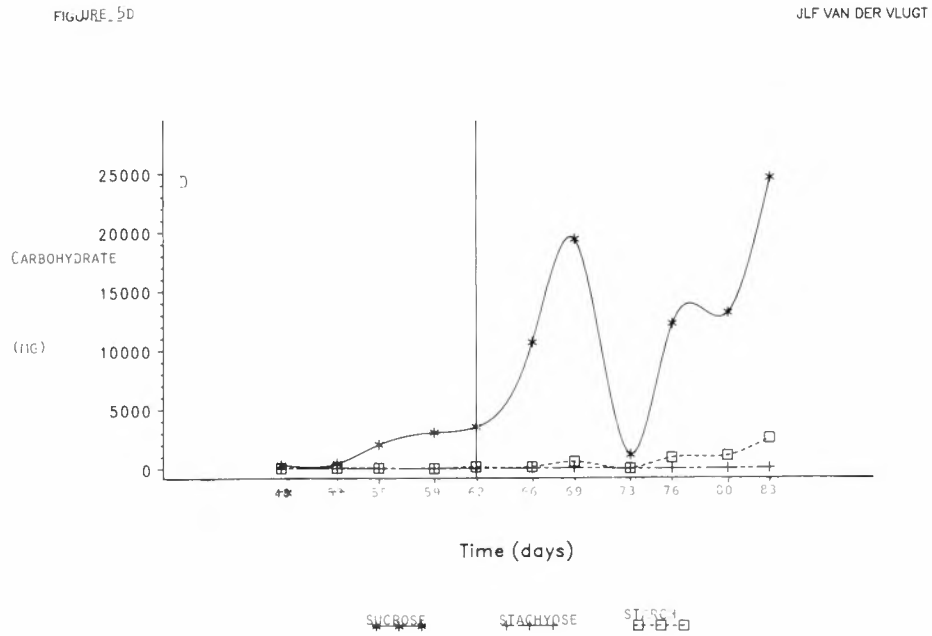


Fig. 5. Content (mg) of different carbohydrates in different plant parts of the cucumber in relation to root death (day 62). A. Carbohydrates in the root B. Carbohydrates in the top C. Carbohydrates in leaves 4 + 5 D. Carbohydrates in the fruit

the root. Transport of minerals was also reduced because of lack of energy.

Deficiencies could develop in the shoot, since the fruit was also an effective sink for minerals. The plants reacted to the deficiencies by increasing their root growth; an accumulation of starch was seen at the end of the experiment.

The whole process began after flowering but reached its climax when the first fruits were harvested. Then the fruit production rate came into the linear phase of the sigmoidal growth curve.

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VII. THE VERDICT: FRUITS ARE ACCESSORY TO ROOT DEATH. EXUDATES ARE THE CAUSE?

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Vlugt J.L.F. Van der, 1990. VII. The verdict: fruits are accessory to root death. Exudates are the cause? Norwegian Journal of Agricultural Sciences 4: 91-94. ISSN 0801-5341.

In earlier reports it was shown that fruits compete with the other plant parts for assimilates. However, since incomplete or complete removal of the fruits does not prevent root death, competition for assimilates is not considered to be the primary cause of root death.
Root exudates also became suspects as an outcome of these experiments. The literature on root exudates shows that they may be toxic to the plants themselves or to other plant species. The amount and the composition of the exudates vary with growth and development of the plants. The root exudates from the cucumber may be autotoxic, the amount of exudate increasing after flowering.
It is concluded that exudates are the likely cause of root death in most cases. A heavy load of fruits or unfavourable growing conditions may cause root death by promoting competition for assimilates in addition to exudation. Therefore, in winter and spring crops of cucumber root death is more severe than in a summer crop.

Key words: cucumber, *Cucumis sativus*, root exudates.

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In this series two factors have been mentioned as causes of physiological root death in the cucumber (*Cucumis sativus* L.): 1. competition for assimilates between roots and fruits, and 2. root exudation.

From the time of constant fruit production rate, the growth rate of the fruits was such that competition occurred, i.e. after harvesting of the first fruit root death caused by competition could be expected (Van der Vlugt, part V in prep.). On other occasions, when root death occurred before harvesting or even in vegetatively growing crops, exudation was assumed to be the cause of root death (Van der Vlugt, part III, Van der Vlugt, 1986, 1987).

My experiments were directed towards the effects of fruit growth. In this paper they were re-examined with regard to root exudates. The literature on exudates was reviewed to find the possible role of exudates in root death.

INDICATINS OF THE ROLE OF EXUDATES IN ROOT DEATH

The results from our experiments confirm the impression that root death is less severe in summer than in winter. Light conditions are better in summer, so

competition is not expected to be as significant a factor as in winter. Another factor must therefore be involved in root death, presumably root exudates.

In the high nitrogen treatment (Van der Vlugt, 1987) relatively fewer fruits were expected than in the low nitrogen treatments; the assimilate supply to the roots would be larger, therefore the roots would also exude more. Root death occurred before harvesting in this treatment. Pegg (1986) showed that tomato roots exuded more when the assimilate supply was larger.

The same effect was shown in different ways in the cucumber experiments with different fruit loads and different plant density (Van der Vlugt, 1986; Van der Vlugt, part III). Root death occurred in all treatments, more plants and fewer fruits giving a higher concentration of exudates. It must be assumed that *Cucurbita* roots are less susceptible to exudates or exude less than cucumber roots.

In the experiments with wide gullies and with containers a large volume of nutrient solution was available to each plant (Van der Vlugt, parts IV, V and VI). In this way it would take a longer time for toxic concentrations of exudates to be reached than in commercial installations. In this case the fruits would be more important. It was found that vigorous root growth was an advantage in these experiments. A vigorously growing root system is probably a good competitor if the exudate concentration is kept low.

OTHER EFFECTS OF ROOT EXUDATES

Root exudates have been investigated in connection with weed growth and crop rotation. Auto-intoxication has been directly shown for the asparagus (Young, 1984). New plants have difficulties in becoming established in an asparagus field. This has been shown indirectly for wheatgrass. Bokhari & Singh (1974) obtained more plant material from plants which had been cut than from control plants. In the cut plants they assumed less material to have been exuded from the roots, and therefore no inhibition of root growth when needed for the regrowth of the aerial part of the plant.

In *Cucumis* spp. and sweet pepper plants, which were kept vegetative, root growth stopped after a while and root death symptoms could be seen (Van der Post, 1968; Hall, 1977). It may be assumed that root exudates caused this root death.

ROOT EXUDATE: QUANTITY AND COMPOSITION

Many seemingly contrasting results have been obtained in experiments with root exudates. The composition of the root exudate varies with the plant growth stage (Vancura & Hanzlikova, 1972; Caussanel & Kunesch, 1979). The effects of root exudation on other plant species depends on the combination of species and plant growth stage of the exudate donor (Pope et al., 1985). A larger amount of exudates gives greater inhibition (Caussanel & Kunesch, 1979).

In several experiments attempts have been made to remove exudates and their effect. Abdel Rahman & Newton (1984) successfully used activated carbon to prevent root death in the tomato. In our experience with cucumber this was not successful. In another experiment with lettuce and other crops Stevens & Tang (1985) found that the hydrophilic substances in the exudate from *Bidens pilosa* were the most toxic.

Caussanel & Kunesch (1979) studied the composition of the exudate of the common lambsquarters (*Chenopodium album*) and its effect on the growth of maize seedlings. They observed that oxalic acid was the active component as

in many other C3-plants and that the amount of oxalic acid increased considerably after flowering of the weed.

The nutrient solutions from the growth analysis experiment (Van der Vlugt, part V in prep.) were stored at 5°C in the dark for 1 year and used for exudate analysis. The exudates had accumulated from day 24. Seeds of cucumber and maize were germinated on filter paper in Petri dishes with 2 x 2 ml distilled water at room temperature; 4 days and 5 days respectively after sowing, the primary roots were measured and the seedlings transferred to 2 x 2 ml of the different nutrient solutions; 8 cucumber and 10 maize seedlings respectively per dish. After 3 days and 5 days respectively the roots were measured again and the increase calculated relative to the original length, since the original length varied both within and between Petri dishes (Table 1). The nutrient solutions were titrated, with oxalic acid used as a standard. The results showed an increase in titratable acids up until root death. The growth of the seedlings seemed to be stimulated at first, but then decreased at higher acid concentrations. It seemed that acids in the cucumber root exudate had a growth inhibiting effect at high concentrations. This inhibitory effect was less severe than with 5 meq/1 of oxalic acid, which completely inhibited growth of both species.

EXUDATION IN NFT

The nutrient solution in NFT (nutrient film technique) is different from that in soil in that the concentration of nutrients is much higher in NFT. Root exudation in itself may be less in NFT because there are no abrasive soil particles to cause wounds and thereby more exudation (Hale & Moore, 1979). The NFT gullies form a closed system in which exudates may accumulate. Low concentrations of exudates may have a stimulating effect (Caussanel & Kunesch, 1979), but the concentration will increase to toxic levels. A larger volume of

Table 1. Amount of titratable acids in the nutrient solutions taken from cucumber plants 27-83 days old. The plants flowered on day 38, the first fruits were harvested on day 55, and root death occurred on day 62. Length of cucumber primary root after 3 days in the different nutrient solutions relative to their original length. Length of maize primary root after 5 days in the different nutrient solutions relative to their original length.

Day	Titratable acids(meq./l)	Cucumber roots	Maize roots
27	1.6	0.9	4.3
31	1.8	1.1	8.9
34	2.5	3.5	7.9
38	2.2	1.9	6.6
41	3.2	1.6	9.7
45	2.1	1.4	8.5
48	3.1	1.1	5.4
52	4.4	1.3	6.6
59	4.9	1.5	5.1
62	5.0	1.2	6.1
66	4.9	1.0	7.3
69	6.3	0.8	2.5
73	2.9	1.1	5.4
76	3.1	0.9	6.2
80	1.0	1.2	10.9
83	1.2	1.4	-

nutrient solution per plant will delay the onset of toxicity.

The role of micro-organisms in exudation is little understood. In NFT a new situation probably exists, with few naturally occurring organisms in the beginning and after a while a microflora which may or may not be different from that in soil. The micro-organisms may use plant exudates and stimulate or inhibit exudation from the plants. Different organisms have different effects and differences between soil and NFT may occur. These factors have had very little investigation so far (Hale & Moore, 1979; Pegg, 1986).

CONCLUSION

Root death is caused either by competition for assimilates or by root exudation. The latter is considered to be the most significant. The former is only of significance under disadvantageous growing conditions. Both factors occur in a natural situation, their combined strength determining the severity of root death. If it were possible to manipulate exudation or build-up of exudates in NFT, then vegetable production in NFT would have great possibilities.

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