Effects of Nitrogen Fertilization and Maturation Conditions on Protein Aggregates and on the Breadmaking Quality of Soissons, a Common Wheat Cultivar[1]

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ABSTRACT

Formation of storage proteins and their aggregation in response to available nitrogen and maturation conditions were investigated for the French wheat cultivar Soissons. N-fertilization level greatly influences the accumulation of the different classes of storage and metabolic proteins by modifying the relative composition of the protein pool, which can be expressed by a decrease of the ratio of glutenins to gliadins and also by variations in baking strength. Changes in glutenin content, high molecular weight glutenin subunits (HMW-GS), and aggregates were monitored by nitrogen determination, sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, size-exclusion high-performance liquid chromatography, and reversed-phase high-performance liquid chromatography. Whereas the composition in HMW-GS remained unchanged, the aggregation level of glutenic polymers developed differently depending on the maturation conditions. These aggregation variations are discussed in connection with the specific contribution of some HMW-GS to the formation of both soluble and insoluble SDS-glutenic aggregates and also with baking strength response.

Nitrogen nutrition is largely considered as the main factor affecting storage proteins as well as the technological quality of wheat samples. According to many studies (Timms et al 1981, Martin et al 1992, Peltonen and Virtanen 1994, Peltonen 1995), both the sources of N fertilization and the application techniques affect the amount of storage proteins. However, to our knowledge, no studies except Scheromm et al (1992) have demonstrated the relationship between N fertilization and the amount of polymeric fractions and type of aggregation of the proteins. Nevertheless, the amount of polymeric fractions has proved to be important in explaining the variation in flour quality (Huebner and Wall 1976, Bietz 1984, Dachkevitch and Autran 1989). Further reports have suggested that baking quality depends on the amount of polymeric fractions (Payne et al 1987, Sutton 1990), including unextractable polymeric proteins (sodium dodecyl sulfate 2% insoluble fraction [F_i]) (Gupta et al 1993).

All the important phenomena that occur during grain filling depend not only on nitrogen fertilization but also on the maturation conditions (temperature and water disposability). Variation of these maturation conditions during this sensitive period (especially after the milky stage) can disturb both protein accumulation and protein aggregation kinetics (Kasarda 1989, Randall and Moss 1990, Triboi 1990). All these effects can potentially modify the technological quality of wheat flour by increasing or masking the influence of nitrogen supplies.

The aim of the present work is to assess cultivar response (modification of the main classes of storage proteins ratios, evolution of both the aggregation level of glutenin polymers and the specific contribution of the high molecular weight glutenin subunits [HMW-GS] to their formation) under various nitrogen fertilization conditions. To introduce natural modifications of maturation conditions that can be respectively assimilated to limitations of nitrogen and water disposability for grain filling, we used both unfertilized and untreated controls (growth and maturation without fungicides). Using nitrogen determination, size-exclusion high-performance liquid chromatography (SE-HPLC) and reversed-phase high-performance liquid chromatography (RP-HPLC) as well as the alveograph test, we investigated both the quantitative variations of the physicochemical parameters and the variations in breadmaking quality of flour.

MATERIALS AND METHODS

Field Experiments

Soissons was the only bread wheat cultivar used. It was selected on the basis of its commercial importance in the French wheat market and its relative variability in breadmaking quality (Martin and Taureau 1992). This cultivar was grown at the experimental farm of INRA Auzeville (1992–93) on a deep silty-clay loam, with an organic matter content of 1.6% (0–30 cm). This experiment was part of studies on cultural rotations (Debaeke and Hilaire 1991). The cultivated area was divided into 57 fundamental plots of 540 m^2 that underwent a particular agronomic management (previous cultivation, nitrogen soil level). Each plot was divided into four subplots of 105 m^2 that corresponded respectively to one fertilized control (T), to an unfertilized control (ON), to a fertilized control without any protection against fungi (SF), and to a fertilized control without any herbicide treatment (SH). Three of the four controls were used for this study (T, ON, SF). The N fertilization was selected to correspond to the different current usage for wheat production in the southwest of France. This program yielded a series of samples with increasing flour protein concentrations (flour protein varied between 7 and 14%). Because of the great number of plots to study, 15 × 3 significant plots (T, ON, and SF) were chosen for technological tests, HMW-GS composition, and quantification. They are representative of the 57 × 3 general plots.

Sample Preparation

Flour samples (240 mg) were stirred for 2 hr at room temperature in 30 ml of 0.1*M* sodium phosphate buffer (pH 6.90) containing 2% (w/w) SDS. These extractions were followed by a denaturation of the proteases (5 min at 90°C) and by centrifugation during 30 min at 12,500 × *g* at 20°C in a Jouan centrifuge (model

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during 30 min at 12,500 × g at 20°C in a Jouan centrifuge (model MR 1822). Clear supernatants (soluble fraction, F_s) were immediately frozen at −18°C. Pellets (insoluble fraction, F_i) were freeze-dried and the protein insoluble fraction was determined by nitrogen analysis. Three or four replicates were done and combined for analysis.

Nitrogen Determination

The Dumas method (AOAC 7.024) was used to determine the N concentration of the dry samples (flour, lyophilized F_i). Protein concentration was determined on a Perkin Elmer apparatus (model PE 2410 series II) by multiplying N values by 5.7.

SE-HPLC

The SE-HPLC apparatus was a ABI model that includes a pump model 400 and a diode array detector model 1000S. A TSK 4000-SW size exclusion analytical column (7.5 × 300 mm, 450Å, TOSOHAAS, Stuttgart, Germany) protected by a guard column (7.5 × 75 mm, 250Å) was used. A 0.1M sodium phosphate buffer (pH 6.90) containing 0.1% (w/w) SDS was used as eluent with a flow rate of 0.7 ml/min as previously described (Bietz 1984, Dachkevitch and Autran 1989). During the fractionation, the column was thermostated at 25°C. The frozen supernatants were heated at room temperature (22°C) and then filtered through a 0.45-µm nylon membrane (C.I.L.). Clear supernatant (20 µl) was applied to the column using a loop injector. The column effluent was monitored at 214 nm and 0.1 absorbance units full scale; chromatograms were analyzed using Labcalc software (Galactic Industries). The major peaks (F_1 to F_4) were eluted between 9 and 20 min as described previously (Dachkevitch and Autran 1989). The first fraction (F_1) should correspond to highly aggregated material (mainly glutenins) and eluted at the void volume of the column. Fraction 2 (F_2) eluted as proteins of 100–520 kDa and should consist of aggregates smaller than those of F_1. Fractions 3 and 4 (F_3 and F_4) corresponded essentially to monomeric proteins (gliadins and salt-soluble proteins, respectively). Apparent molecular weight of major peaks were estimated by calibrating the column with four unreduced protein standards: thyroglobulin (669,000), bovine serum albumin (66,000), chymotrypsinogen (25,700), and cytochrome C (12,400). All quantitative data are expressed both on a relative basis (percentages of total area of chromatograms) and on an absolute basis (amount of proteins). There is indeed a very strong linear relationship between the amount of soluble proteins (P_s), estimated by the difference between the amount of total protein in flour (P_f), and the amount of protein in the residue of SDS extraction (P_i), and the total area of the chromatogram for each flour sample ($r = 0.90$*** where *, **, and *** are significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; $n = 165$).

A Pharmacia SE-FPLC system (Pharmacia-Biotech S.A., 78280, Gyancourt, France) was used with a Superose-6 prep grade gel-filtration column (HR 16/50) to characterize the HPLC fractions from flour extracts. Analysis was performed with the same buffer for elution and protein extraction, with a 2 ml/min flow rate and a 500-µl sample loading. Twelve fractions containing protein material were collected and could be assigned respectively to the regions F_1 to F_5 of the analytical elution curve (Fig. 1).

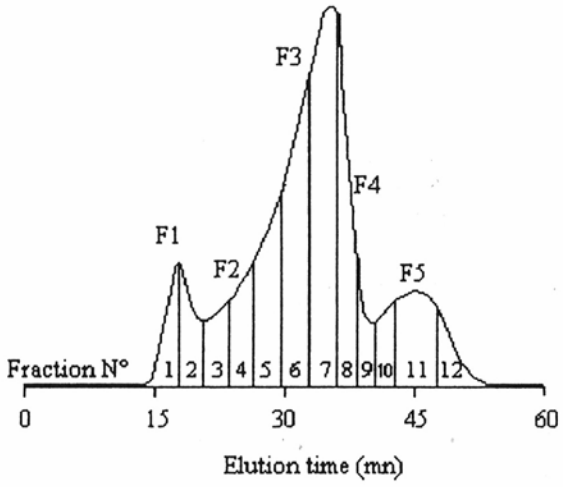

Fig. 1. Typical elution pattern of concentrated wheat storage proteins extracted by phosphate-sodium dodecyl sulfate buffer (pH 6.9) on a Pharmacia Superose-6 prep grade gel-filtration column.

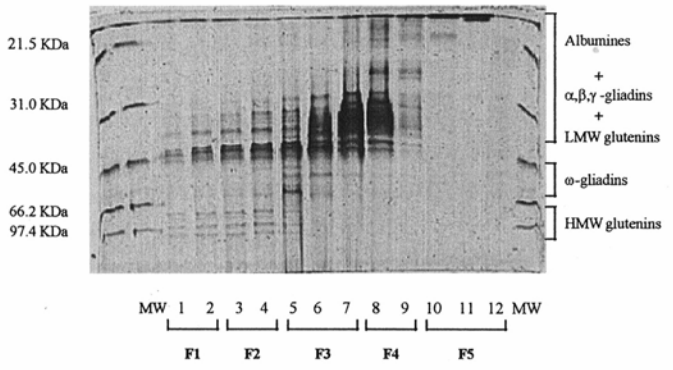

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns of reduced protein fractions of Soissons wheat collected from Superose-6 prep grade gel-filtration column. F1–F4, analytical size-exclusion high-performance liquid chromatography peak number. MW = molecular weight reference mixture. Molecular weight markers: phosphorylase b (97.4 kDa); bovine serum albumin (66.2 kDa); ovalbumin (45.0 kDa); bovine carbonic anhydrase (31.0 kDa); soybean trypsin inhibitor (21.5 kDa); hen egg white lysozyme (14.4 kDa).

Electrophoresis

To recover concentrated proteins from collected peaks, the SDS was removed by precipitating the protein fraction with 15% (w/v) trichloroacetic acid according to Dachkevitch (1989). The protein residue was washed three times with 5 ml of acetone and dried under vacuum. Dry protein extracts were dissolved by a reducing solvent (1M Tris-HCl buffer, pH 6.8, 2% [w/v] SDS, 10% [v/v] glycerol, 0.05% [w/v] bromophenol blue, and 5% [v/v] 2-mercaptoethanol) and were incubated at 100°C for 5 min. Reduced proteins were then electrophoresed according to Laemmli (1970) in vertical SDS-polyacrylamide gel electrophoresis slabs (Mini-PROTEAN II cell, Bio-Rad Laboratories) at a gel concentration of 13% in a discontinuous, pH 6.8–8.8, Tris-HCl-SDS buffer system (Payne et al 1979). Gels were stained for 1 hr and 45 min

TABLE I
Gradient Programs for Separation of Reduced Gliadins-Glutenins and Alkylated High Molecular Weight Glutenin Subunits by Reversed-Phase High-Performance Liquid Chromatography

	Gradient Composition (% acetonitrile)		
Time of Segments (min)	Start	End	Type[a]
Program A			
0.00–1.50	24	24	Isocratic
1.50–52.50	24	48	Linear
52.50–54.00	48	48	Isocratic
54.00–54.50	48	24	Linear
54.50–60.00	24	24	Isocratic
Program B			
0.00–1.50	24	24	Isocratic
1.50–28.50	24	35.5	Linear
28.50–29.00	35.5	48	Linear
29.00–29.50	48	48	Isocratic
29.50–30.50	48	24	Linear
30.50–34.00	24	24	Isocratic

[a] Flow rate = 1 ml/min.

with 0.1% (w/v) Coomassie blue R-250 in fixative (40% MeOH [v/v], 10% acetic acid [v/v]) (Fig. 2).

Extraction and Precipitation of HMW-GS

Flour or F_i samples (1.0 g) were extracted successively with 4.0 ml and 3.0 ml of 50% (v/v) propan-1-ol containing 1% (w/v) dithiothreitol (DTT) by sonication for 2 min at power setting 50% (output 25W, 23 kHz) using a stepped microtip probe (3 mm diameter) followed by continual stirring for 30 min at 60°C. After centrifugation (10,000 × *g*, 10 min), the first and second extracts were pooled. Precipitation, resolubilization, and alkylation of HMW-GS were performed as described previously (Marchylo and al 1989).

RP-HPLC

A Spectra-Physics HPLC and a PC1000/Spectranet data and chromatography control station (Spectra-Physics analytical software, San Jose, CA) in conjunction with a Zorbax 300 SB-C8 column (C8, 300-Å pore size, 5-µm particle size, 15 cm (4.6 mm i.d. [Supelco Inc., Bellefont, PA]) preceded by a guard column of the same packing material (Zorbax 300 SB-C8, 2-cm (4.6 mm, i.d.) were used for analysis of storage proteins. Column temperature was maintained at 50°C; the column effluent was monitored at 210 nm. Storage proteins were resolved using gradient program A (60 min), whereas alkylated HMW-GS were eluted with a gradient program B (34 min) as described in Table I. Injection volumes were 15 µl for all analyses, except for HMW-GS, for which 30-µl injection volumes in conjunction with a multiple 5-µl injection technique (Marchylo and Kruger 1988) were used.

Technological Tests

Baking strength was determined on the *W* index (Chopin alveograph, Tripette et Renaud) according to standard 5530/4 (IOS).

Statistical Analysis

The Stat-ITCF (ITCF, France) computer package was used for correlation analyses based on linear regression.

RESULTS AND DISCUSSION

Maturation Conditions and Variation of Flour Protein Composition with Protein Content

Table II lists some of the agronomic information that characterizes the different plots T, ON, and SF. The number of heads per square meter (NE/m^2) gives us an idea of the structure of the different populations. our results show that because of its direct action on tillering, the nitrogen fertilization causes a notable increase of the density of heads (from 370 to 520 heads/m^2). The grain number per head is slightly different between reference plots and others plots (ON and SF). The kernel weight (KW) is an essential parameter for the grain yield and is controlled by climatic conditions (water supply, temperature) (Grignac 1981, Rellier 1981, Triboï et al 1985). For instance, the plots that underwent water limitation during the grain filling period (SF) are characterized by very low KW (19.74 mg/kernel). The ON plots have significantly higher KW than the T plots (32.08 vs. 26.85 mg/kernel). This difference of KW between the plots T and ON indicate more important filling speeds (Triboï et al 1985). As the filling speed is highly dependent on the amount of assimilates temporarily stored in vegetative parts, the plots with a low number of heads per square meter were therefore the most favored. The grain filling speed is correlated with the quantity of water, which remains constant between the milky stage and the desiccation stage (Geslin and Vernet 1952). During this period, the water quantity entering the kernel is compensated by the evaporation (root absorption ≈ E.T.P.) (Fig. 3), and products of chemosynthesis accumulate in the kernel.

The quantities of the different groups of proteins increase in proportion to the total protein content of the various flours. Effectively, whether it concerns glutenins (Fig. 4A), gliadins (Fig. 5A), or albumins and globulins (Fig. 6A), their respective evolutions are positively related to the total protein content (FP). A lower nitrogen reserve for the ON plots reveals a weaker accumulation of glutenins, gliadins, globulins and albumins. In the same way, after a fungic attack (SF), we found a more important amount of glutenins, gliadins, albumins and globulins in flour due to an indirect effect of concentration. These observations are in total agreement with the results of Doekes and Wennekes (1982) and of Branlard et al (1985).

The relative contribution of each group of proteins to the FP shows different behaviors in harmony with the nature of proteins but also with the conditions of nitrogen application and maturation. So, the variations of the relative percentage of glutenins (35–43% for T and ON, respectively) do not seem to be related to the FP evolutions (Fig. 4B). However, the SF treatment introduces a

TABLE II
Effect of N Fertilization and Fungicide Protection on Agronomic Traits of Soissons

Treatment[a]	Grain Number (grains/head)	Kernel Weight (mg/kernel)	Heads/m^2
T	34.85a[b]	26.85b	517.51a
ON	30.65b	32.80a	367.85b
SF	29.42b	19.74c	524.17a

[a] Fertilized control (T), unfertilized control (ON), fertilized control without any protection against fungi (SF).
[b] Means followed by same letter are not significantly different at $P = 0.05$, according to Newman & Keuls test. Readings are means of 57 plots.

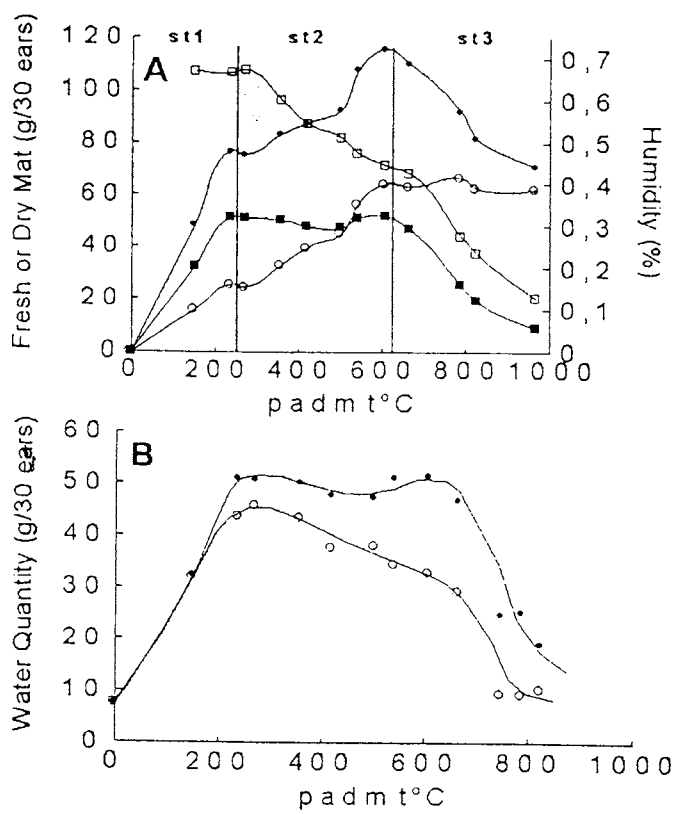

Fig. 3. Grain filling period of Soissons wheat (plot C5T). **A:** Evolution of head humidity (□); water quantity (■); head dry matter (○); and fresh matter (●) as a function of the sum of the daily mean temperature (base 0°C) postanthesis. Stages: st1, cell division; st2, *palier hydrique*; st3, dehydration. **B:** Evolution of water quantity (plot C5) of a control with (●) or without (○) protection against fungi as a function of the sum of daily mean temperature.

supplemental variability (30–45%). On the contrary, the relative percentage of gliadins (Fig. 5B) is very well correlated to the FP. This behavior has already been observed for many genotypes (Gupta et al 1992). Yet, after a modification of the maturation conditions (SF), we can observe a decrease of the relative percentage of gliadins compared with the reference plots. The T plots are characterized by a postanthesis nitrogen absorption estimated at 20% of the total nitrogen absorbed at maturity (unpublished data). The relatively less important accumulation of gliadins of the SF plots might come from the limitation of this late absorption phenomenon. Finally, the portion represented by the metabolic proteins diminishes when the FP increases (Fig. 6B). It is interesting to observe that albumins-globulins, which are commonly associated to a decrease of dough strength, are more important for the SF plots. These results confirm those obtained by Fullington and Nityagopal (1986). The variation of the ratio of glutenins to gliadins in flour (Fig. 7) corresponds to various behaviors of synthesis induced either by different levels of nitrogen availability (T and ON) or by a limitation of water disposability (T and SF). This ratio, often used as a quality indicator (Doekes and Wennekes 1982, MacRitchie 1989, Martin and al 1992), tends to decrease as the protein content increases. However, if the protein increase is the result of an important limitation of starch accumulation, the previous relation does not exist because the modification of the gliadins accumulation appears to be more affected than that of the glutenins in these conditions (Fig. 5B).

Variations of Glutenin Solubilization and Molecular Weight Distribution in SE-HPLC with Protein Content (Consequences on *W*)

It is likely that SE-HPLC combined with N analysis is a more accurate tool than sequential extraction to investigate the glutenic aggregation. This aggregate level can be evaluated by comparing F_2, F_1, and F_i fractions, which are respectively representative of glutenin fractions with higher and higher molecular weight and lower and lower SDS solubility. The F_1 / F_2 ratio and the F_i quantity have already been considered as good indicators of soft wheat qualitative potential (Dachkevitch and Autran 1989). Figure 8A and B show that the quantitative evolution of the different glutenins fractions is related to the accumulation of the storage proteins. The increases of $F_1 + F_2$ fraction is nevertheless limited in their progress and reach upper limits represented by the T treatments. This limitation phenomenon does not exist in the case of the SDS-2% insoluble fraction (F_i) (Fig. 8A). On the other

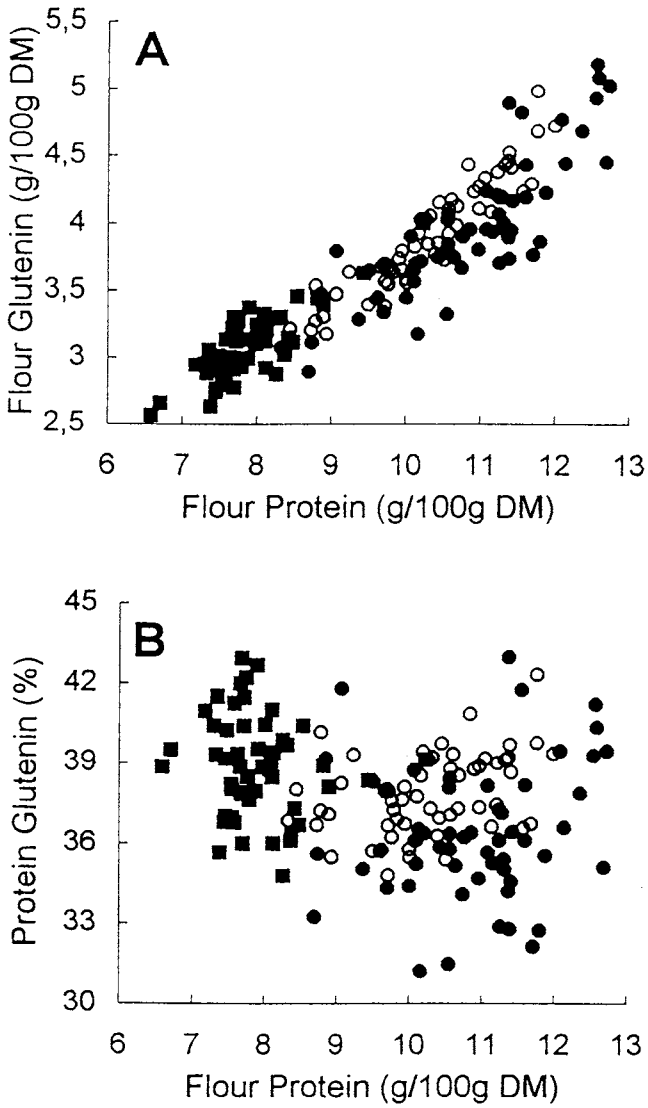

Fig. 4. Percent glutenin in flour (**A**) and protein (**B**) as a function of percent protein in flour from fertilized control plots (○), unfertilized control plots (■); and fertilized control plots without any protection against fungi (●). A: $r = 0.923$ ($P < 0.001$, $n = 165$). B: $r = -0.251$ ($P < 0.01$, $n = 165$).

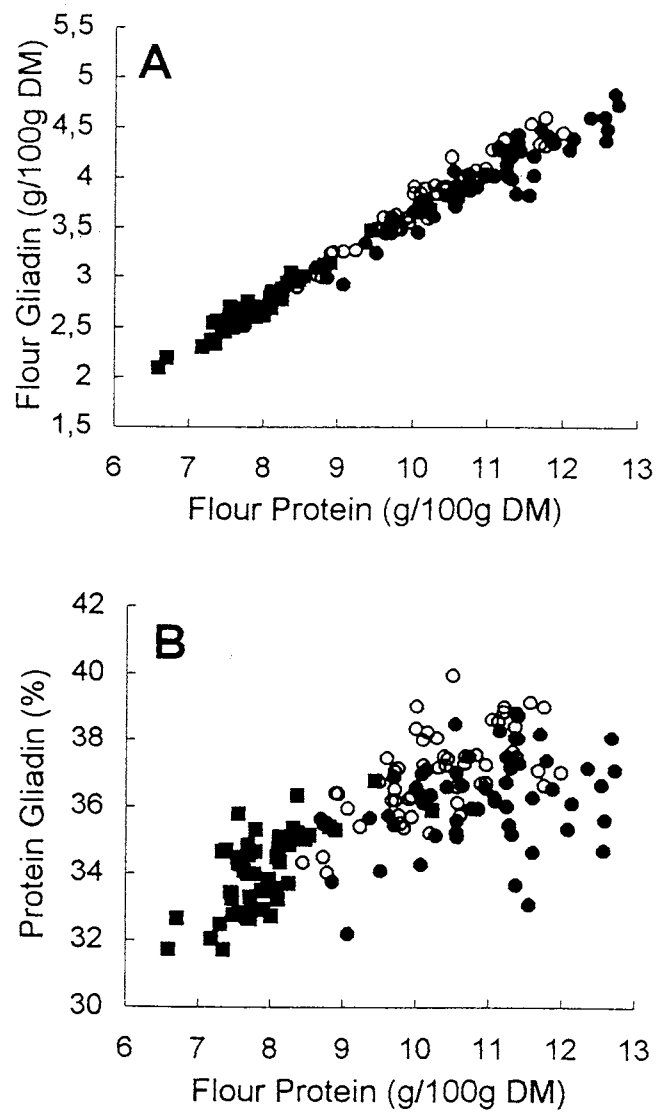

Fig. 5. Percent gliadin in flour (**A**) and protein (**B**) as a function of percent protein in flour from fertilized control plots (○); unfertilized control plots (■); and fertilized control plots without any protection against fungi (●). A: $r = 0.983$ ($P < 0.001$, $n = 165$). B: $r = 0.720$ ($P < 0.001$, $n = 165$).

hand, we can observe a less important relative accumulation of the F_i for the SF plots. The SDS-solubility of the glutenin aggregates for a cultivar such as Soissons seems to be strongly affected by the nutrition conditions during grain filling.

For each treatment, correlations are calculated between baking strength (W index) and protein content, flour gliadin, and flour glutenin (Fig. 9A–C). The results show that the W index is significantly linked to flour protein and flour glutenin for all plots. Several differences can be observed between the SF, T, and ON treatments. Variations in gliadin, glutenin, albumin and globulin compositions are responsible for part of these differences. For example, a decrease in water disposability during the grain filling, resulting in a fungic attack (SF), as shown previously, induces a fall in gliadin accumulation. On the contrary, an artificial limitation of storage protein accumulation (ON) has consequences of limiting breadmaking strength.

However, it seems that clear divergence of the regression lines between SF plots and T plots (Fig. 9A) may be mainly attributed not to the variations of the gliadin content of the flours (Fig. 9B), but rather to a difference in solubility of the glutenin aggregates (Fig. 9C). SF plots are characterized not only by a weaker accumulation of gliadins, but also by a higher proportion of SDS soluble glutenin aggregates (Fig. 8B). Khan and Bushuk (1978) have been evoking the existence of a relationship between the breadmaking quality of wheat and the acetic acid insoluble glutenin aggregates. In our experience, the conditions of maturation may modify the breadmaking quality of one precise genotype, by modifying the solubility of these glutenin aggregates.

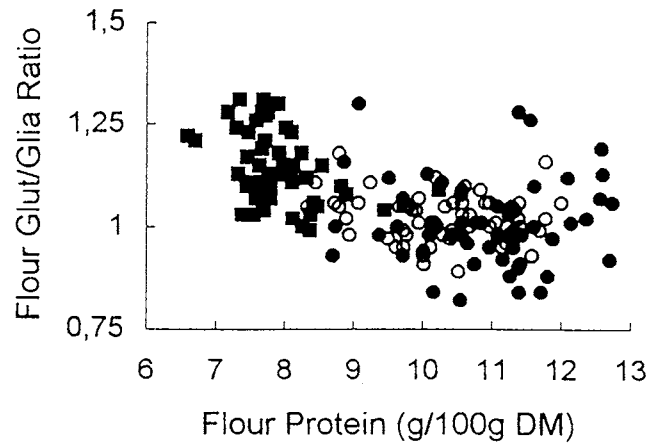

Fig. 7. Flour glutenin-to-gliadin ratio as a function of percent protein in flour from fertilized control plots (○); unfertilized control plots (■); and fertilized control plots without any protection against fungi (●). r = −0.533 ($P < 0.001$, $n = 165$).

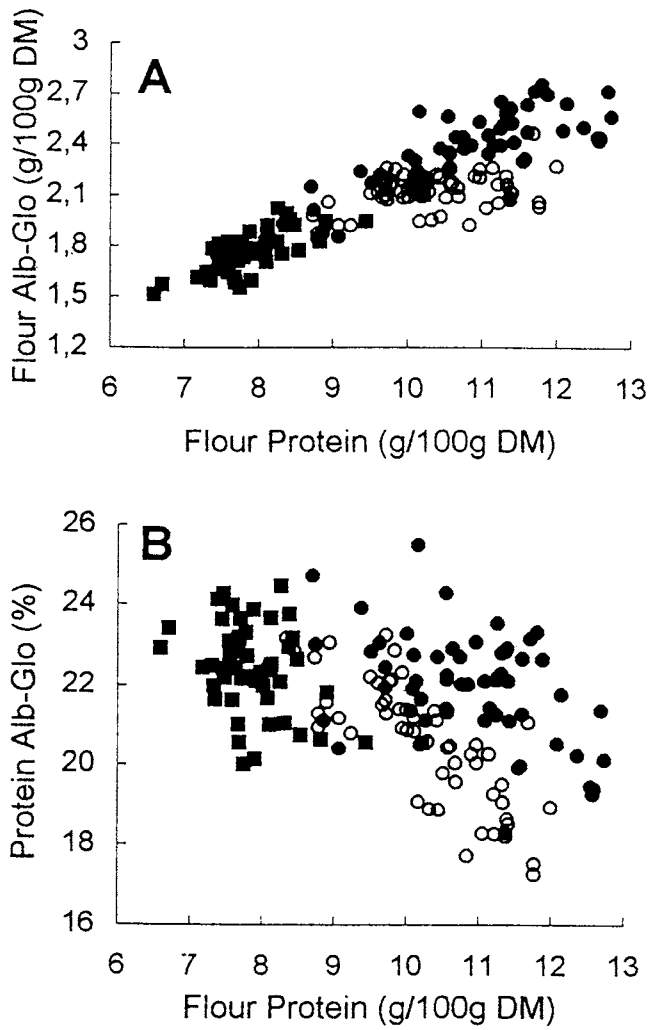

Fig. 6. Percent albumin-globulin in flour (**A**) and protein (**B**) as a function of percent protein in flour from fertilized control plots (○); unfertilized control plots (■); and fertilized control plots without any protection against fungi (●). A: $r = 0.878$ ($P < 0.001$, $n = 165$). B: $r = -0.486$ ($P < 0.001$, $n = 165$).

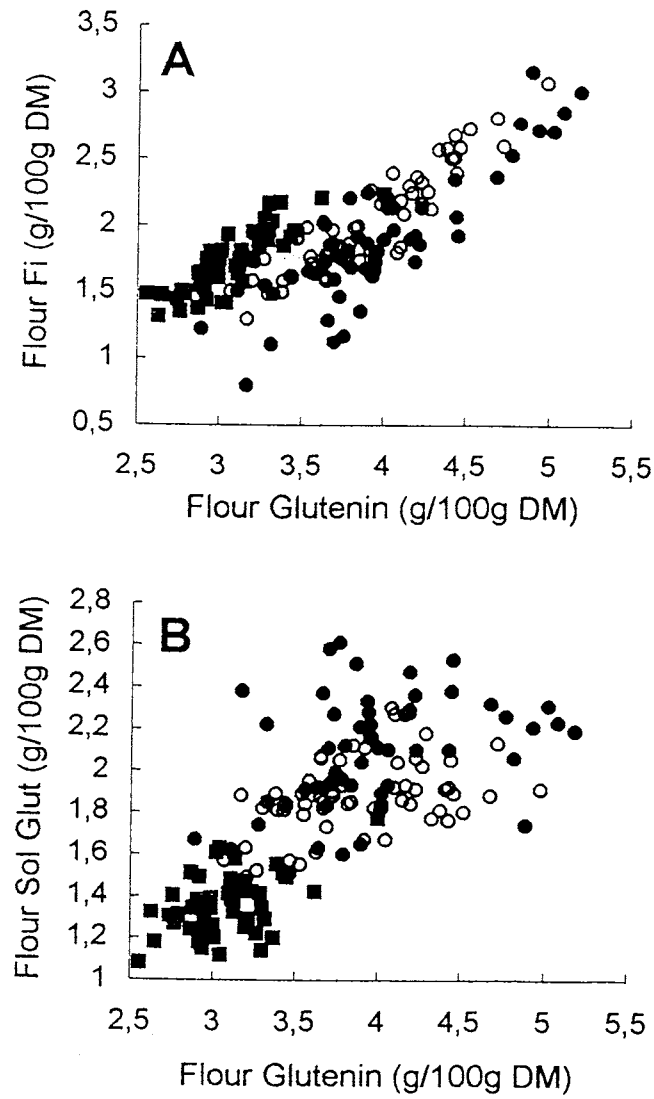

Fig. 8. Percent F_i in flour (**A**) and percent soluble glutenin ($F_1 + F_2$) in flour (**B**) as a function of percent glutenin in flour from fertilized control plots (○); unfertilized control plots (■); and fertilized control plots without any protection against fungi (●).

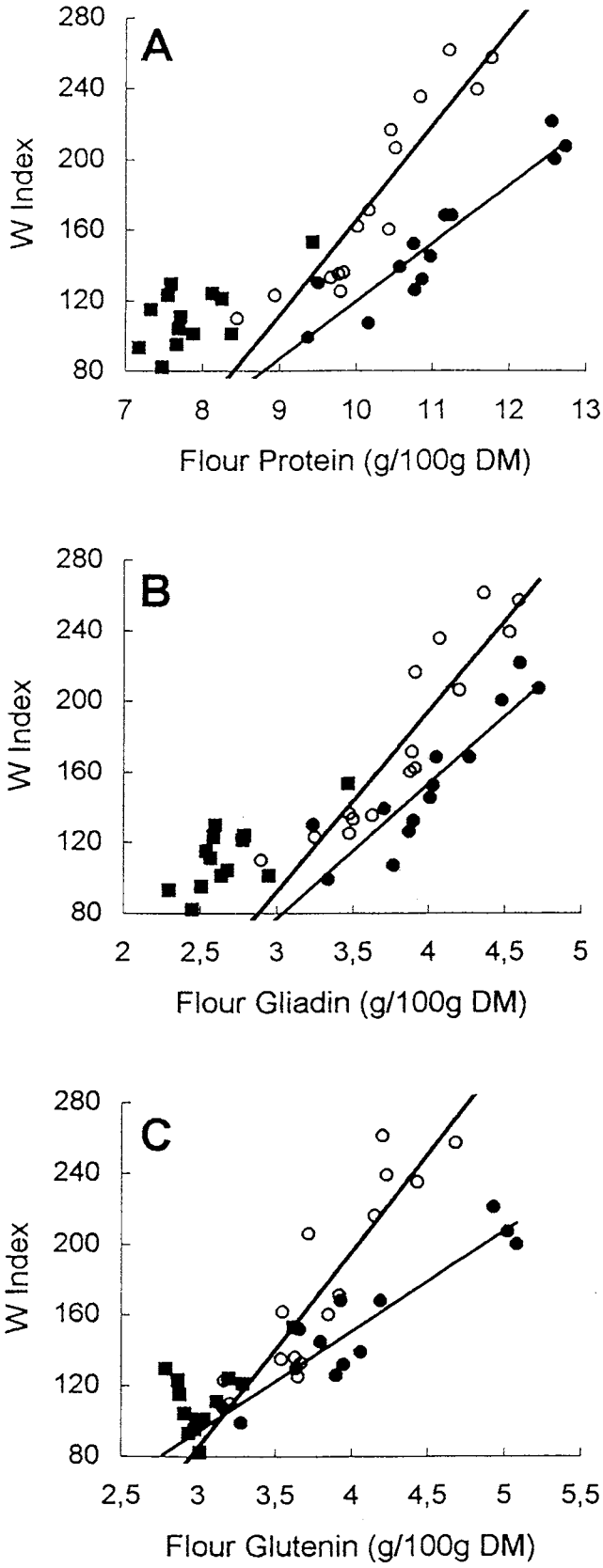

Fig. 9. Correlation between: **A,** flour protein (N × 5,7); **B,** flour gliadin; and **C,** flour glutenin with alveograph *W* index for Soissons wheat. Fertilized control plots (O); unfertilized control plots (■); and fertilized control plots without any protection against fungi (●). A(O): $r = 0.917$ ($P < 0.001$, $n = 15$). A(●): $r = 0.933$ ($P < 0.001$, $n = 13$). B(O): $r = 0.931$ ($P < 0.001$, $n = 15$). B(●): 0.8947 ($P < 0.001$, $n = 13$). C(O): 0.898 ($P < 0.001$, $n = 15$). C(●): $r = 0.928$ ($P < 0.001$, $n = 13$).

Variation of Total and Individual HMW-GS with Glutenin Content in Flour and in Insoluble Fraction (F_i)

The total amount of HMW-GS extracted from the flour differed considerably between the plots. In agreement with the results of Kolster et al (1991), an increase in the glutenin content resulted in an increase of the total amount of HMW-GS (Fig. 10B). There is a very strong linear relationship ($r = 0.976$***) between F_i glutenin content and the total quantity of HMW-GS accumulated in the F_i (Fig. 10A).

The next stage of our work was to separate quantitatively the F_i HMW-GS extracted from the flour to determine their respective contribution to the formation of the insoluble aggregates. Figure 11A–J give a more detailed idea of the relative distribution of the five HMW-GS in setting the flour against the F_i. In flour, certain HMW-GS are quantitatively more abundant than others: subunits 7 and 5 are the most numerous with 30 and 27%, respectively, of their content in HMW-GS, whereas subunits 10 and 2* are clearly less abundant with 17 and 16%, respectively; and finally, subunit 8 had only 10%. These percentages are very stable in spite of the variations of N nutrition (ON and T) and despite a water deficiency (SF). However, we did note certain perceptible tendencies. For example, the decrease in percentage of subunit 5 can be compared to the increase in percentage of subunits 7 and 2* (Fig.

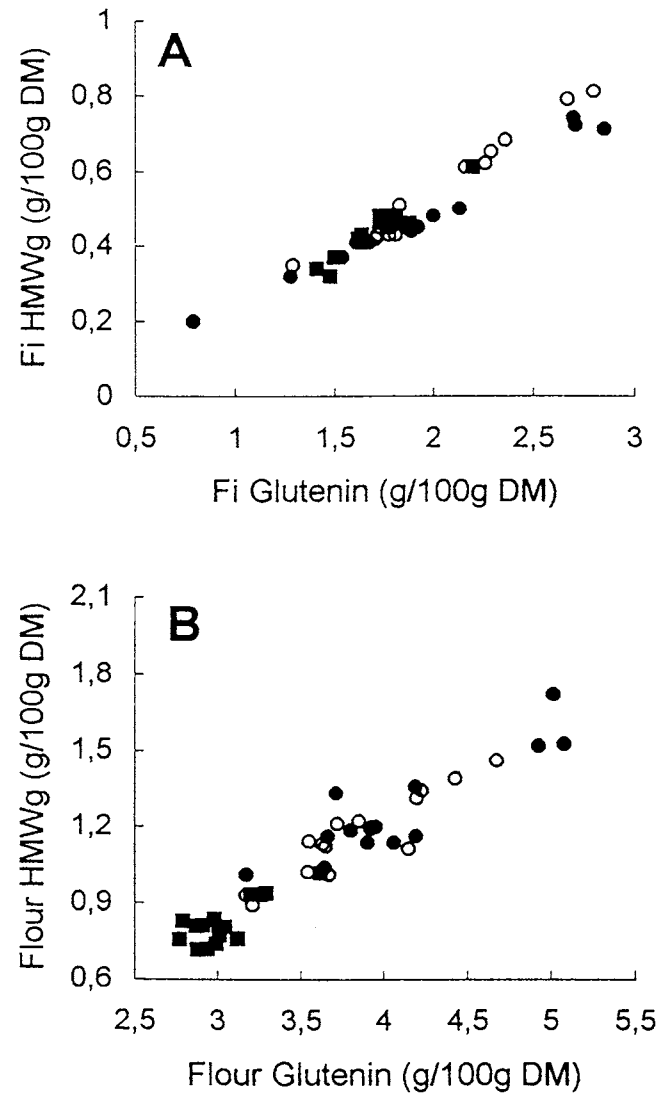

Fig. 10. Percent high molecular weight glutenin subunits (HMW-GS) in flour (**A**) and F_i (**B**) as a function of percent glutenin in flour from fertilized control plots (O); unfertilized control plots (■); and fertilized control plots without any protection against fungi (●). A: $r = 0.950$ ($P < 0.001$, $n = 45$). B: $r = 0.970$ ($P < 0.001$, $n = 45$).

11C, G, and I) according to the total amount of HMW-GS accumulated in the flour. The relative proportions of HMW-GS in F_i follow the same hierarchy as the one observed for flour. The main characteristic of these proportions is the stability with which they settle. However, certain subunits are sensitive to specific treatments. Subunits 5 and 2* (Fig. 11D and J) had a fungal attack during the grain filling period that resulted in a significant decrease (5–7%) and increase (3–5%), respectively, in the percentage of the subunits considered.

The insoluble glutenin aggregates are slightly richer in subunits 10, 5, and 2* and, on the contrary, poorer in subunits 7 and 8. The variations in proportion of the subunits 5 (–5 to –7%) and 7 (+5 to +7%) seem to particularly characterize these extracts. These observations are in agreement with all the results obtained by Autran and Morel (1990). Therefore, it seems that small but real differences in the incorporation of HMW-GS occur within these aggregates.

CONCLUSIONS

The nitrogen fertilization is the source for both the yield and the quality formation for soft wheat. The N fertilization level influences the accumulation of the different classes of storage and metabolic proteins by modifying the relative composition of the protein pool. The accumulation of gliadins becomes more important than that of glutenins, and the albumins and globulins are less and less represented as the flour protein increases. This phenomenon is established for the two populations: T and ON. Therefore, the N fertilization intervenes not only as a quantitative parameter but also as a qualitative one (glutenin-to-gliadin ratio). The glutenins, and more especially the different types of aggregates, considered as quality predictors, are also exposed to several qualitative variations directly related to the N fertilization. The solubility (SDS-2%) degree of the aggregative fractions ($F_1 + F_2$) reaches a maximum due to the nitrogen nutrition effect, even though, meanwhile, the F_i accumulation continues. In addition, the nitrogen fertilization level influences the insolubilization level of glutenin aggregates for the cultivar Soissons.

The maturation conditions, mainly responsible for the KW elaboration, also appear to be very important for the protein balance. The limitation of water nutrition, resulting from a pathogenic attack, leads to a limitation of the accumulation of the storage proteins (Fig. 4B and 5B) for the benefit of the metabolic proteins (Fig. 6B). When the pathogenic attack is important, the accumulation of gliadins is more affected, as shown by the variations in the glutenin-to-gliadin ratio (Fig. 7). Furthermore, the stressed plots (SF) are characterized by a greater solubility of glutenin aggregates (Fig. 8B) and a decay in F_i accumulation.

The accumulation of the HMW-GS pool in Soissons flour is very strongly correlated with the accumulation of storage proteins and all the more with the accumulation of glutenins. It is the same at the individual level; there is no significant difference in the behavior between the five HMW-GS. This phenomenon does not seem to be affected by the big fluctuations in maturation conditions. The glutenins present in the aggregate fractions, insoluble to SDS-2% (F_i), have relative proportions in HMW-GS very close to those of whole flour. However, certain HMW-GS (subunits 5, 10 and 2*) contribute a little more than others to forming these insoluble aggregates. On the contrary, subunits 7 and 8 are correlated with soluble fractions. These observations are verified for other genotypes also, as shown by the works of Autran and Morel (1990). Whereas the level of N nutrition acts quantitatively on the equipment of HMW-GS, variations of maturation conditions (SF) act in a certain way on the incorporation rate of certain HMW-GS in insoluble aggregates. We are thus able to observe significant variations as far as SF plots are concerned in the relative proportions of F_i of subunits 5 and 2*. The lack of solubility of glutenin aggregates thus cannot be attributed to a high level of HMW-GS of these aggregates.

These results indicate that the insolubility of glutenin aggregates in Soissons is not the result of a different composition but more the result of a modification of structure of these polymers. The incorporation rate of HMW-GS in the insoluble fractions is not significantly different from that of whole flour, so it is difficult to believe that an increase in the molecular weight of an always linear polymer may act alone against the SDS action. On the contrary, modifying the structure of the aggregates by making it more reticulate (Wall 1979, Lorenzo-Seva 1993) would succeed in modifying considerably the solubility to SDS of these fractions. The conditions in which the grain filling occurs would limit this phenomenon by slowing down the aggregation mechanism. So it seems necessary to investigate more precisely the protein aggregation and insolubilization phenomena.

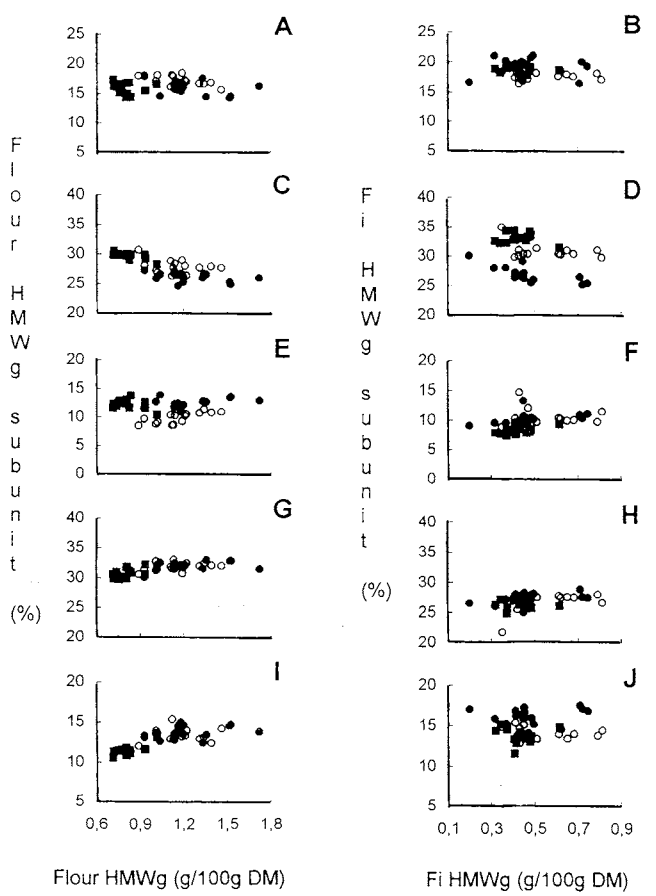

Fig. 11. Percent subunit 10 (**A**), 5 (**C**), 8 (**E**), 7 (**G**), and 2* (**I**) in flour high molecular weight glutenin subunits (HMW-GS) as a function of percent HMW-GS in flour. Percent subunit 10 (**B**), 5 (**D**), 8 (**F**), 7 (**H**), and 2* (**J**) in F_i HMW-GS as a function of percent HMW-GS in F_i. Fertilized control plots (O); unfertilized control plots (■); and fertilized control plots without any protection against fungi (●).

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