

Effect of Aphid Feeding on the Glutenin, Gliadin and Total Protein Contents of Wheat Flour

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Plant sucking aphids cause both quantitative and qualitative yield losses in cereals; moreover aphid-transmitted viruses are responsible for other quantitative and qualitative damages, thus direct or indirect effects of aphid infection are in focus of interest.

Bread-making quality of wheat flour is determined primarily by the protein content and composition, the gluten proteins (glutenins, gliadins) being the prime factors. Allelic composition of the gliadin- and glutenin loci as well as the absolute amount and/or the relative ratio of gliadins to glutenins are very important in dough making and in determining baking quality.

Wheat plants were caged at the beginning of stem elongation. Cages were treated with 0.1% methyl parathion. One week later, the caged plants were artificially infected with 5 alata individuals of *Metopolophium dirhodum*, *Diuraphis noxia*, *Sitobion avenae* and *Rhopalosiphum padi*. Flour from grains originating from plants infected artificially with cereal aphids were analyzed for glutenin and gliadin and total protein content, using Size Exclusion HPLC. It was found that aphid infection had significant effect on the glutenin and gliadin content, the total protein content and the gliadin/glutenin ratio. Both the glutenin and gliadin content was significantly higher in the seeds harvested from aphid infected plants. However, the gliadin/glutenin ratio was significantly lower in wheat flour prepared from aphid infected plants than in those from uninfected control. The most significant decrease in gliadin/glutenin ratio was caused by *M. dirhodum*, *D. noxia*, *S. avenae* infection followed by *R. padi* at high-abundance and low-abundance, respectively. As the gliadin/glutenin ratio was significantly lower in flours made from aphid infected wheat seeds, it may be suggested that aphid feeding results in decreased bread making quality of wheat flour.

Keywords: cereal aphids, *Metopolophium dirhodum*, *Diuraphis noxia*, *Sitobion avenae*, *Rhopalosiphum padi*, SE-HPLC, glutenin, gliadin, total proteins.

Aphids are most abundant in the temperate region where main cereal growing areas are situated, therefore cereals from all over the world are attacked by aphids (Blackman and Eastop, 1984). Apart from direct feeding damage (sucking plant sap) aphids act as vectors of phytopathogenic viruses which cause further quantitative and qualitative damages on cereals (Hoffman and Kolb, 1997). In terms of yield losses resulting from cereal aphid feeding on wheat, *Schizaphis graminum* Rondani and *Rhopalosiphum padi* L. are reported

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to be more damaging at similar population densities than *Sitobion avenae* F. (Kieckhefer and Kantack, 1988). *S. graminum* causes chlorosis due to loss of chlorophyll content of the infected leaves, apparently caused by a toxin injected by the feeding of *S. graminum*, (Girma et al., 1999) and results in significant reductions in plant biomass. Yield losses of 35 to 60% have been recorded in greenbug-damaged wheat (Kieckhefer and Kantack, 1980, 1988). Kuroli and Németh (1987) reported 33–65% yield loss of winter wheat due to autumn infestation of *R. padi*. The Russian wheat aphid *Diuraphis noxia* Kurdjumov was described to cause significantly greater yield loss than *S. graminum* (Gellner et al., 1990). *D. noxia* feeding having a marked effect on the membranes and chloroplasts, so the latter membranes disintegrated and their contents spread through out the cell and chloroplasts disappeared. *R. padi* and *S. graminum* did not cause disintegration of chloroplasts (Fouché et al., 1984). However, *Sipha flava* (Forbes) feeding resulted in similar changes in chloroplast structure such as its swelling, loss of grana structure and increase of the number of starch granules on *Sorghum halepense* (L.) (González et al., 2002).

As a result of quality control measures being in use since 1860, Hungarian wheat is notable for its good overall features (Pollhamer, 1981). Bread-making quality of wheat flour is determined primarily by its protein content and composition as it was summarized by Mac Ritchie (1984) and much effort has been made to elucidate which protein constituents are responsible for specific quality differences. Gluten proteins are the prime factors governing wheat properties (Finney, 1943; Mac Ritchie, 1978). Originally, the gluten proteins were divided into two main classes according to their solubility in ethanol+water (70+30, by volume); soluble proteins were classed as gliadin and insoluble proteins as glutenins (Osborne, 1907). The distinction between solubility classes is not sharp enough and the definition of these classes based on their molecular size seems to be a better approach. Polymeric glutenins are proteins formed from glutenin subunits through inter-molecular disulphide bonds. They are larger than 100 kDa, those between 100 and 25 kDa are mainly gliadin and proteins smaller than 25 kDa were defined as albumin and globulin (Meredith and Wren, 1966; Bushuk and Wrigley, 1971). The subunits of glutenins can be divided into two groups: high molecular weight (HMW) (Mac Ritchie et al., 1990) and low molecular weight (LMW) glutenin subunits (Shewry et al., 1992). According to an other and widely accepted approach the wheat gluten proteins are distributed into three main groups: the S(sulphur)-rich prolamins, the S-poor prolamins and the High Molecular Weight (HMW) prolamins (Shewry and Tatham, 1990).

Size-exclusion High Performance Liquid Chromatography (SE-HPLC) has been successively used for the study of cereal storage proteins, particularly in wheat. This methodology reliably and reproducibly separates the three main classes of wheat storage proteins: glutenins, gliadins and albumins+globulins (Singh et al., 1990a, b; Batey et al., 1991; Gupta et al., 1993). It is quite evident that the above qualities have been studied extensively by food scientists and plant breeders, and results in this area are well documented (Mac Ritchie, 1978; Shewry and Tatham, 1990). Infection by cereal aphids is also in the focus of interest in the plant protection field (Rabe et al., 1989).

In the present publication we compare the glutenin, gliadin and albumin+globulin contents of flours from plants artificially infected with cereal aphids indigenous to

Hungary with those of flours from plants infected with *D. noxia*. Macroscopic features and evaluation of quality markers were used to identify qualitative and quantitative changes related to dough making and to the baking quality of wheat flour resulting from aphid feeding on the wheat plants from which the different flours originated. The results, obtained by HPLC (SE-HPLC), allowed calculation of gliadin/glutenin ratios, comparison of total protein contents.

Materials and Methods

Artificial aphid infection

Metopolophium dirhodum Walker, *Diuraphis noxia* Kurdjumov, *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* L. cereal aphid species used for artificial aphid infection were reared in the greenhouse on potted barley (*Hordeum vulgare* L. cv Pannonia).

Winter wheat (*Triticum aestivum* L.) cultivar 'MV 17' (Hungary) was sown on 28th October 2000 at a seed rate of 220 kg ha at the experimental plot of the Plant Protection Institute of the Hungarian Academy of Sciences, Nagykovácsi, Hungary. Two hundred plants were selected randomly and caged individually, using a fine mesh cloth to block aphid emigration and immigration, on 25th April 2001 at a growth stage of 25–30 tillering and beginning of stem elongation (Tottman and Broad, 1987). Each cage covered an area of 450–500 cm². Caged plants were situated in the middle of the cages. The height of the cages was 90–100 cm. Plants, typically having 10–12 tillers, were randomly chosen for caging. Plants after caging were sprayed with 0.1% methyl-parathion to kill aphids responsible for natural infection, then cages were closed.

A week later 60 caged plants were artificially infected with 5 aptera individuals of *M. dirhodum*, *D. noxia*, *S. avenae*, respectively. Hundred-twenty caged plants were infected with *R. padi* L., and 30 caged plants were left uninfected. Half of the aphid-infected cages were used for aphid population assessment and the other half was used for quality studies. One month after caging *R. padi* infected cages were opened cages were labeled high or low according to the *R. padi* density. Six replicates from each category: *M. dirhodum*, *D. noxia*, *S. avenae* and *R. padi* high and low densities and uninfected control caged plants were kept for grain harvest. One replicate consisted of 5 cages.

Aphid population assessment

The plants were sampled destructively at weekly intervals over the six weeks from 11th June 2001 (GS 39, flag leaf collar just visible) until 9th July 2001 (GS 87, hard dough, ripening). Five plants from *M. dirhodum*, *D. noxia*, *S. avenae* and *R. padi* infected cages were sampled at each sampling date. Plants were transferred to Berlese funnels for 5 days to collect aphids. Aphids were extracted from the funnels into 70% aqueous ethanol and the numbers of different species were counted.

Harvesting for flour quality studies

Heads from *M. dirhodum*, *D. noxia*, *R. padi* high-and low-densities and *S. avenae*-infected and uninfected cages (six replicates as above) were collected separately at GS 93 (caryopsis loosening in daytime). Grain from each cage was kept together as one sample for further analyses, yielding a total of 180 samples.

Processing of grain and milling

Following harvest, length of the heads was measured, kernel number/head was counted, seeds were removed from heads and 30 randomly selected kernel-weight was measured from each treatment. The seeds from each cage were stored in respective bags and separately milled. An FQC-Micro scale labmill (Technical University, Budapest, Hungary) was used to grind the seeds. Flour was separated with a 150–200 µm sieve and the fraction falling between 150–200 µm was used for SE-HPLC.

Quantification of polymeric proteins by SE-HPLC

A modified version of methods described by Singh et al. (1990a) and Batey et al. (1991) was used to determine the glutenin, gliadin and albumin+globulin content of the samples by SE-HPLC. The flour samples (10 mg per Eppendorf tube) were mixed with sodium dodecyl sulphate (SDS, 5 g litre⁻¹; 1 ml) in 0.05M sodium-phosphate buffer (pH=6.9). The mixture was first gently shaken for 10 min to dissolve soluble proteins (gliadins), followed by a one-minute sonication (ensuring that the sample was completely dispersed within the first few seconds) for liberation of nonsoluble polymeric proteins (glutenins) to give a total polymeric and monomeric protein extract including albumins and globulins. The extract was centrifuged (10 min; 14,000 rpm) and the supernatant was filtered quantitatively through a 0.45 µm Millex PVDF filter (Millipore, Bedford, U.S.A). Aliquots of 20 µl from each extract were injected into a Beckman HPLC system comprising of two pumps (Model 110A), an injector/organizer (Model 340), a controller (Model 420), a UV-visible detector (System Gold Programmable Detector Module 166) and a Shimadzu C-R3A integrator for data acquisition. A Zorbax BIO GF-450 Size-Exclusion column equipped with a Diol preparative guard column (Agilent Technologies, U.S.A) was used for analysis. The column was eluted with HPLC grade acetonitrile + HPLC grade water (50+50, by volume) containing trifluoroacetic acid (1 ml litre⁻¹ at 2 ml min⁻¹) flow rate for 10 min. and proteins were detected at a wavelength of 214 nm. From each flour sample (180) the above described extraction was performed and two parallels were run.

The elution profile was divided into three main peaks corresponding to polymeric proteins, containing mainly glutenins, the gliadins and the albumins+globulins, respectively. The effect of aphid infestation on the amount of respective macro-proteins and total proteins was calculated on the basis of the previously deduced concentration value multiplied by a generated kernel weight correction factor (kernel weight(g)/0.033628(g); where the denominator is the average of all measured kernel weights) which is proportional to the kernel weight. The obtained result represents the amount of different protein classes in individual kernels.

In this study the respective and total protein integrated peak areas were subjected to statistical analysis.

Statistical analysis

Analysis of variance was used to prove the effect of different aphid species on the length of the heads, kernel number/head, weight of the kernels and the qualitative characters as glutenin, gliadin and the albumin+globulin contents of the flour samples (the average of two parallels were taken), as well as the gliadin/glutenin ratio using the integrated areas under the peaks for the proteins obtained by the SE-HPLC procedure. Tukey HSD test was used for post hoc tests for comparison of mean values. Analyses were made using the STATISTICA 6.0 program package (Statsoft, Inc. 2003).

Results

Artificial aphid infection

Aphid population was the highest on 18th June regardless of the aphid species (Fig. 1). *R. padi* formed the most abundant colonies on plants where *R. padi* populations were high, the lowest aphid density was recorded in *R. padi* low density cages. *S. avenae* proved to be the second most abundant species, followed by *M. dirhodum* and *D. noxia*.

The mean of *R. padi* individuals on the *R. padi* High labeled plants were 134.6/aphids/tiller on 18 June, followed by 55.6 *S. avenae*/tiller, 35.1 *M. dirhodum* /tiller, 25.0

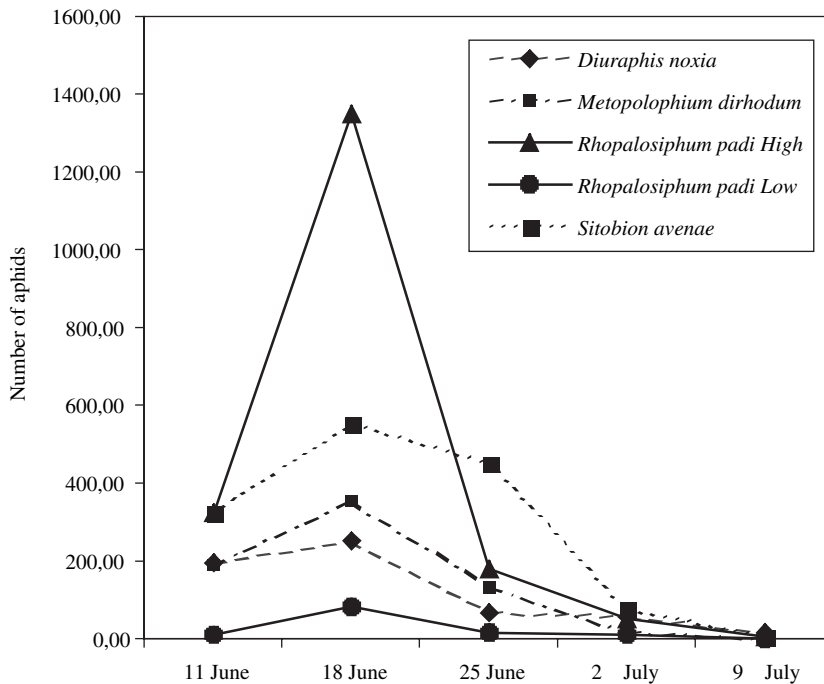


Fig. 1. The mean number of aphids per cage on the artificially infested plants

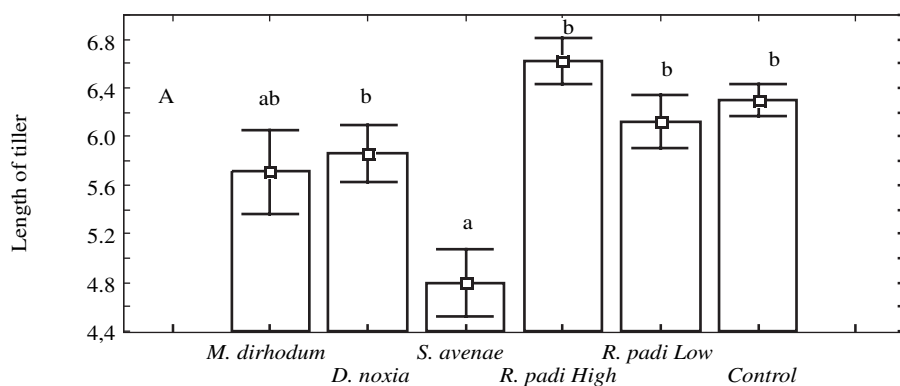
D. noxia individuals/tiller, and the mean value of *R. padi* individuals on the Low labeled plants were 8 aphid/tiller at peak population.

Damage caused by aphids

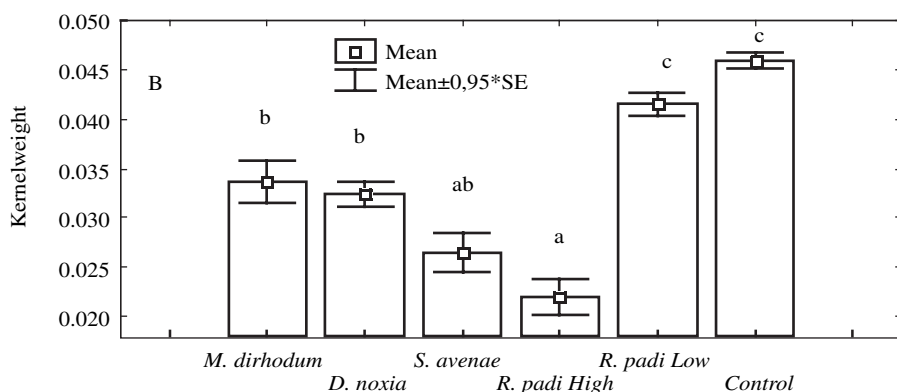
Analysis of variance revealed the significant effect of the aphid infection on the length of the heads, number of kernels/head, kernel weight and the qualitative characters as glutenin, gliadin, albumin+globulin and the total protein contents of the flour.

Quantitative characters

Mean head length varied between 2.5 and 10.5 cm, while mean kernel number/head varied between 14 and 65, the mean kernel weight varied between 0.011 and 0.054 g (Fig. 2a). Only *S. avenae* infection resulted in significant decrease in head length. However,



Length of tiller: $F(5;174) = 6.074$; $p = 0.000$



Kernel weight: $F(5;174) = 28.337$; $p = 00.000$

Fig. 2. Mean length of tillers and mean kernel weight of uninfected plants and that of infected by *M. dirhodum*, *D. noxia*, *S. avenae* and *R. padi* High and Low numbers.

Different letters represent significant differences ($P < 0.05$) between groups by Tukey post hoc analyses

apart from *R. padi* Low infection level other aphid species and *R. padi* High-density significantly decreased the weight of individual kernels (Fig. 2b).

Qualitative characters

The glutenin content of the wheat flour significantly grew due to *D. noxia*, *S. avenae*, and *R. padi* feeding (Fig. 3a). The gliadin content of the flour also significantly increased in response to aphid feeding damage regardless of the species (Fig. 3b). The gliadin/glutenin ratio, however, significantly decreased due to aphid feeding damage, except when *R. padi* was present on the isolated plants in Low-density (Fig. 3c). Albumin content of the flour was significantly different from the control when flour was acquired from *M. dirhodum* damaged kernels (Fig. 3d). Total protein content of the

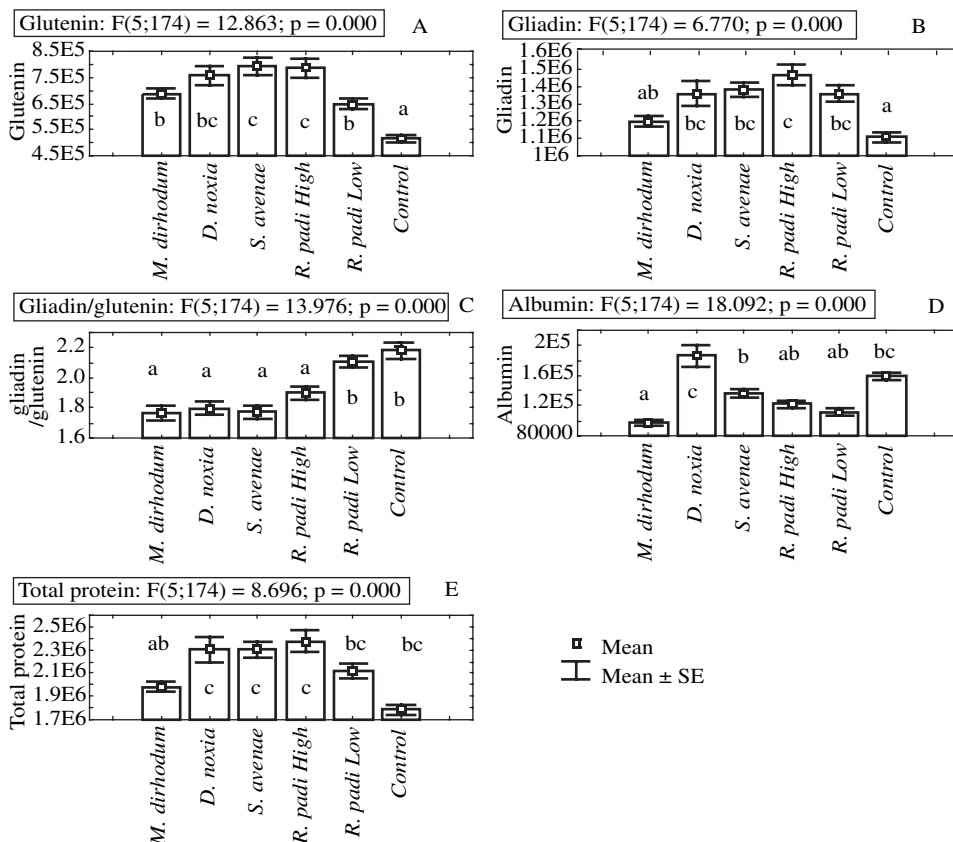


Fig. 3. Effect of aphid infection on the protein content of winter wheat flour. The values on y axis represent the integrated values of HPLC peaks obtained by processing 10 mg of flour by samples (data are mm² for A, B, D, E and ratio data for C). The upper boxes show the results of one way ANOVA-s on the effect of aphid infection groups on protein content of the flour. Different letters represent significant differences (p<0.05) between groups by Tukey post hoc analyses

flour significantly increased compared to the control in flour originated from kernels damaged by *D. noxia*, *S. avenae*, *R. padi* both High- and Low-densities (Fig. 3e).

Qualitative composition in respect to individual kernel gave a somewhat different result, as summarized in Fig. 4, but well correlates with individual kernel weights (Fig. 2b). The absolute amount of glutenin was significantly lower in case of *S. avenae* and *R. padi* High, while as for gliadin only *R. padi* High-infestation lowered the amount significantly. Albumin and globulin amount per kernel was significantly lower in all infestation cases. Total protein per kernel also decreased in all cases except in *R. padi* Low-infestation.

Relationship between yield characters

There was significant correlation between the length of the heads and the number of kernels/head, weight of kernels/head and mean kernel weight (Table 1). There was a close correlation between the mean kernel weight and the weight of kernels/head, glutenin and gliadin content of the flour, gliadin/glutenin ratio and the total protein content of the flour (Table 1). A negative relationship between weight of kernels and glutenin, gliadin and total protein contents of the flour was found.

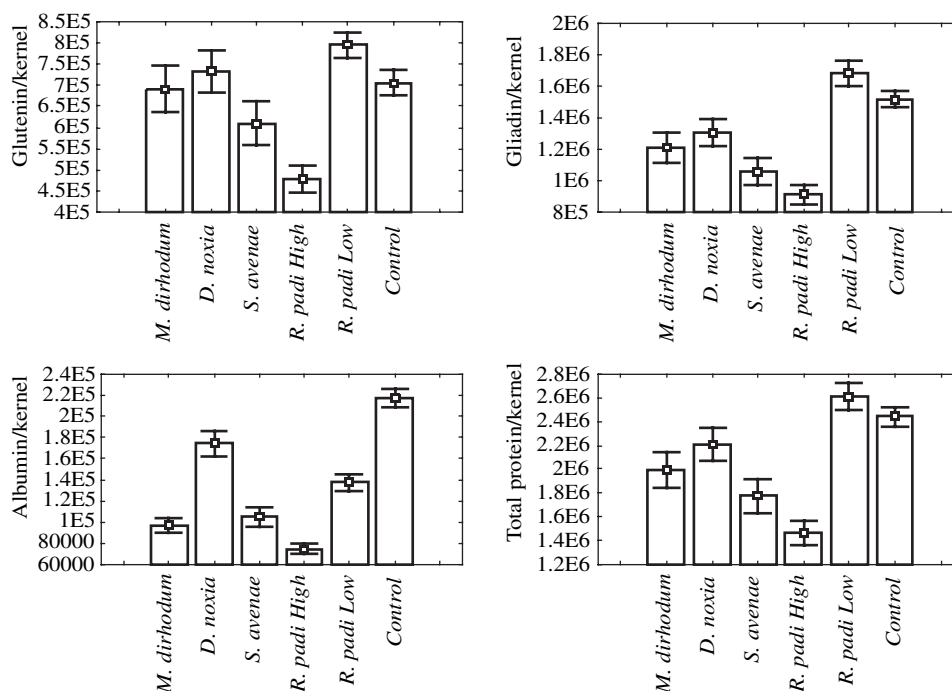


Fig. 4. Effect of aphid infection on the protein content of winter wheat kernels. The values on y axis represent the integrated values of HPLC peaks obtained by processing 10 mg of flour by samples multiplied by the mean kernelweight in the given sample. The upper boxes show the results of one-way ANOVA-s on the effect of aphid infection groups on protein content of the flour. Different letters represent significant differences ($p < 0.05$) between groups by Tukey post hoc analyses

Table 1

Correlations between qualitative and quantitative yield parameters

Variables	Length of heads	Kernel number/head	Kernel weight/head	Mean kernel weight	Glutenin	Gliadin	Albumin + globulin	Gliadin/glutenin
Kernel number/head	$R^2=0.90$ $P=0.00^*$							
Kernel weight/head	$R^2=0.74$ $P=0.00^*$	$R^2=0.85$ $P=0.00^*$						
Mean kernel weight	$R^2=0.31$ $P=0.00^*$	$R^2=0.34$ $P=0.00^*$	$R^2=0.72$ $P=0.00^*$					
Glutenin	$R^2=0.41$ $P=0.58$	$R^2=-0.02$ $P=0.74$	$R^2=-0.26$ $P=0.00^*$	$R^2=-0.42$ $P=0.00^*$				
Gliadin	$R^2=0.14$ $P=0.058$	$R^2=0.09$ $P=0.22$	$R^2=-0.11$ $P=0.11$	$R^2=-0.28$ $P=0.00^*$	$R^2=0.76$ $P=0.00$			
Albumin + Globulin	$R^2=-0.12$ $P=0.09$	$R^2=-0.23$ $P=0.00^*$	$R^2=-0.19$ $P=0.00^*$	$R^2=-0.06$ $P=0.41$	$R^2=0.07$ $P=0.29$	$R^2=0.13$ $P=0.06$		
Gliadin/glutenin	$R^2=-0.13$ $P=0.06$	$R^2=-0.18$ $P=0.013^*$	$R^2=0.29$ $P=0.00$	$R^2=0.32$ $P=0.00^*$	$R^2=-0.51$ $P=0.00^*$	$R^2=0.12$ $P=0.09$	$R^2=0.07$ $P=0.33$	
Total protein	$R^2=0.09$ $P=0.21$	$R^2=0.23$ $P=0.75$	$R^2=-0.20$ $P=0.00^*$	$R^2=-0.35$ $P=0.00^*$	$R^2=0.89$ $P=0.00^*$	$R^2=0.96$ $P=0.00^*$	$R^2=0.23$ $P=0.00^*$	$R^2=0.11$ $P=0.12$

(*marked correlations are significant at $P < 0.05$, $N=180$)

Discussion

The aim of this recent study was to further study the effects of aphid feeding on the baking quality of the flour since data are very scarce or practically non existent in the field, and to find correlation between insect infestation and basic wheat/flour quality, the latter being otherwise crucial in bread-making.

It is well known that glutenin quality and quantity governs mixing requirements and dough strength while gliadin quality and quantity is primarily responsible for dough extensibility. The balance of dough strength and extensibility determine loaf volume (Finney et al., 1982). Therefore, the absolute amount and/or the relative proportion of gliadins to the glutenins are critically important in dough making and determining baking quality. The protein content and glutenin to gliadin ratio have different roles in influencing the various dough and bread making parameters. Increase in glutenin to gliadin ratio at fixed protein content increases mixing time, mixograph peak resistance, maximum resistance to extension, and loaf volume. An increase in glutenin to gliadin ratio decreases resistance breakdown and extensibility of dough (Uthayakumaran et al., 1999). The elongation properties of the dough may be influenced in a complex way by altering the glutenin to gliadin ratio, reflecting the different rheological properties of glutenin and gliadin. Adding glutenin increases the rupture viscosity and lowers the rupture strain of dough, while addition of gliadin has the opposite effect. Both findings provide rheological support for the widely accepted interpretation of glutenins contributing the elastic and strength characteristics to the dough, and gliadins, the flow properties (Uthayakumaran et al., 2000a). HMW-

glutenins contribute positively to dough strength to a lower extent. The increase of Low Molecular Weight (LMW)-glutenin adds to extensibility (Uthayakumaran et al., 2000b).

Some previous studies showed considerable yield decrease (30–40%) caused even by the low autumn infestation of *R. padi*, *S. graminum* and *D. noxia* (10–15 aphids per plant) (Kieckhefer and Gellner, 1992). When *S. avenae* abundance reached 20–30 aphids per plant during milk development quite significant yield decrease was reported (Lee et al., 1981). Lee et al. (1981) also demonstrated that *S. avenae* infestation affected not only yield quantity but bread-making quality of flour like color, increased nicotinic acid and thiamine content and decreased HMW-glutenin content. Recently Sivri et al. (2004) demonstrated that total proteins extracted from control and bug damaged samples differed in size distribution of the polymeric protein and their glutenin/gliadin ratios. They supposed that bug protease caused dough weakening by degradation of glutenin, presumably by hydrolysis.

During feeding activity the first thing an aphid does after entering a cell with its piercing-sucking mouthpart is to salivate, because it needs to wet and clean its food receptacle and gustatory sensilla of residue from previous sap sampling or feeding (Harris and Harris, 2001). Enzymes in aphid saliva begin detoxification and digestion of the plant sap before it enters into the aphid alimentary canal. Aphid saliva spreads all over the plant within hours, when aphid feeding begins (Giménez et al., 1997).

The examination of the studied 1800 heads showed that the components of the yield decreasing effect of aphid damage is notable. From the examined cereal aphid species *S. avenae* feeding significantly decreased the length of heads, the number of kernels/head and the weight of kernels/tiller (data not shown). Except in case of the Low-population of *R. padi*, all other species *M. dirhodum*, *D. noxia*, *S. avenae* and *R. padi* High-density significantly decreased the weight of individual kernels.

Cereal aphid species, *D. noxia*, *S. avenae*, *R. padi* High- and Low-densities significantly increased gliadin content of the flour. The glutenin content of the flour also grew significantly regardless of the aphid species. In spite of significant glutenin and gliadin content increase due to aphid feeding the gliadin/glutenin ratio decreased notably regardless of the aphid species. These findings are in good correlation with our previous findings from year 2000, when significant decrease in gliadin/glutenin ratio was reported caused by *D. noxia* infection, followed by *R. padi* and then *S. avenae*, respectively (Basky and Fónagy, 2003). On the other hand, *M. dirhodum* feeding damage resulted in significant albumin and globulin content decrease as well, while total protein content was significantly higher in flour made from *D. noxia*, *S. avenae*, *R. padi* High- and *R. padi* Low-population damaged wheat.

The precipitation was very low during the vegetative period of the wheat, spring and summer of year 2001 were very dry, and therefore the kernels were shriveled even in the uninfected control. The negative relationship between weight of kernels and glutenin, gliadin and total protein contents of the flour indicates that proteins were more concentrated in the smaller kernels. Aphid feeding is known to result in a decreased water status of the damaged plants even at sufficient water supply as it was demonstrated by Carberera et al. (1995). Under dry conditions aphid-sucking activity may cause an even greater damage on infected wheat, than under wet weather conditions, causing a significant decline in general quality in respect to bread-making. This is also in agreement with the finding of Blumenthal et al. (1994), who described that heat stress during grain filling leads to important changes

in the synthesis of gluten proteins. They reported the reduced synthesis of the HMW subunits of glutenin, and continuing synthesis of other gluten proteins, particularly various gliadin proteins. It is however, possible that the stress response could be solely due to aphid feeding as was suggested by Békés (pers. comm. 2005).

Based on these presented results, further, detailed quality studies are required to better understand the synthesis of gluten protein components – indicating that this process is strongly influenced by aphid feeding in cereals. The results of this study clearly demonstrate that scarcely investigated factors of the agro-ecosystems may meaningfully contribute to the yield parameters of the wheat.

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