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Chemical characterization of wheat kernels naturally contaminated by deoxynivalenol-DON when cultivated under nitrogen management strategies[1]

Caracterização química de grãos de trigo naturalmente contaminados por desoxinivalenol-DON quando cultivados sob estratégias de manejo de nitrogênio

Thiago Montagner Souza[2]*, André Mateus Prando[3], Cassia Reika Takabayashi-Yamashita[4], Claudemir Zucareli[2] and Elisa Yoko Hirooka[4]

ABSTRACT - Chemical composition and contamination of wheat kernels (*Triticum aestivum* L.) directly affect the quality of the flour obtained from them, determining its acceptability and use by industry. Field trials were conducted to evaluate the effect of agricultural practices on wheat kernel quality (chemical composition and contamination). Cultivation was carried out following maize or soybean (crop succession), with application of nitrogen doses in topdressing (0, 30, 60, 90, and 120 kg ha^{-1}), using seeds inoculated or not inoculated with *Azospirillum brasilense*. Data were subjected to analysis of variance (ANOVA), comparison of means by Tukey's test (<0.05), and regression to nitrogen doses. Crop succession resulted in changes in kernel chemical composition (p<0.05), with exception for lipid content (p>0.05). Lower protein (-21.6%; p<0.05) and higher total carbohydrate content (+4.5%; p<0.05) were observed when wheat was cultivated after maize, in comparison with soybean succession, and no application of nitrogen in topdressing (0 kg ha^{-1}). When cultivated after maize, protein content in the kernels was the factor with the greatest variation in response to increasing doses of nitrogen (11.1 to 16.5%, an increment up to 48.2% in the total content), due to a positive correlation with the independent variable (r>0.80; p <0.05). In terms of contamination by DON, 83.1% (133/160) of the samples presented contamination below the current maximum tolerated limit stablished by the Brazilian legislation for whole wheat flour (<1250 ug kg^{-1}). Management of nitrogen availability can be recommended as an additional procedure to obtain raw materials with the desired chemical profile.

Key words: *Triticum aestivum* (L.). Crop succession. Nitrogen doses. *Azospirillum brasilense*. Kernel composition.

RESUMO - Composição química e contaminação de grãos de trigo (*Triticum aestivum* L.) afeta diretamente a qualidade da farinha obtida, determinando sua aceitabilidade e uso pela indústria. Ensaios foram conduzidos a campo para avaliar o impacto de práticas agrícolas na qualidade de grãos de trigo (composição química e contaminação). O cultivo foi feito em sucessão ao milho ou soja (sucessão de culturas), com aplicação de nitrogênio em cobertura (0, 30, 60, 90 e 120 kg ha^{-1}), utilizando sementes inoculadas ou não com *Azospirillum brasilense*. Os dados foram submetidos à análise de variância (ANOVA), comparação de médias por teste de Tukey (<0,05) e regressão para doses de nitrogênio. Sucessão de cultura alterou a composição química do grão (p<0,05), com exceção do teor lipídico (p>0,05). Menor teor de proteína (-21,6%; p<0,05) e maior de carboidratos totais (+4,5%; p<0,05) foram observados com o cultivo do trigo após o milho, em comparação a sucessão com a soja e sem aplicação de nitrogênio em cobertura (0 kg ha^{-1}). Quando cultivado após o milho, o teor de proteína nos grãos de trigo foi o fator com maior variação em resposta a doses crescentes de nitrogênio (11,1 a 16,5%, um incremento de até 48,2% no conteúdo total), devido a uma correlação positiva com a variável independente (r> 0,80; p<0,05). Em relação à contaminação por DON, 83,1% (133/160) das amostras apresentaram contaminação abaixo do limite máximo tolerado vigente estabelecido pela legislação brasileira para farinha integral de trigo (<1250 µg kg^{-1}). O manejo da disponibilidade de nitrogênio pode ser recomendado como procedimento adicional para obtenção de matéria-prima com perfil químico desejado.

Palavras-chave: *Triticum aestivum* (L.). Sucessão de cultura. Dose de nitrogênio. *Azospirillum brasilense.* Composição do grão.

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*Author for correspondence
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[1]Parte da Dissertação de Mestrado do primeiro autor apresentada ao programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Londrina/UEL
[2]Departamento de Agronomia, Universidade Estadual de Londrina/UEL, Londrina-PR, Brasil, thiagom@okstate.edu (ORCID ID 0000-0002-8179-8733), claudemircca@uel.br (ORCID ID 0000-0002-5260-0468)
[3]Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Soja, Londrina-PR, Brasil, andre.prando@embrapa.br (ORCID ID 0000-0002-2314-6526)
[4]Departamento de Ciência e Tecnologia de Alimentos, Universidade Estadual de Londrina/UEL, Londrina-PR, Brasil, cassiarty@gmail.com (ORCID ID 0000-0002-4821-1961), hirooka@uel.br (ORCID ID 0000-0003-0481-1188)

INTRODUCTION

In addition to productivity, wheat kernels must have the technological qualities desired by consumers in order to avoid the use of additives, for reasons of cost and food safety (FRANCESCHI *et al*., 2009). The chemical composition of wheat kernels (protein, lipids, minerals, and carbohydrates) determines the technological and nutritional functional characteristics and, together with the structural properties, defines the quality of the flour (SCHEUER *et al*., 2011). In this sense, the most efficient way to achieve high productivity with concomitant high quality is the appropriate application of crop management practices, such as fertilization, predecessor crop selection and inoculation of plant growth promoting bacteria (BENIN *et al*., 2012; BLANDINO *et al*., 2012; PICCININ *et al*., 2013; PINNOW *et al*., 2013; PRANDO *et al*., 2012; VOGEL *et al*., 2013).

The availability of nutrients is a determinant variable in production, with nitrogen as the main limiting factor to development and productivity of the crop, owing to its importance in the formation of amino acids, proteins, chlorophyll, and essential enzymes that stimulate growth and development of the aerial part and root system of the plant (BENIN *et al*., 2012). Because of the effects of nitrogen, nutrient availability in the soil can influence nutritional quality and safety of kernels, as observed by previous authors analyzing maize kernels (SOUZA *et al*., 2016). The nutritional status of plants can also determine their greater or lesser predisposition to diseases, presenting the balanced one greater capacity of defense due to improvements in the main defense mechanisms under a form of physical barrier (CARVALHO *et al*., 2013; TERZI *et al*., 2014). However, even with the nutritional balance effect, one of the most important cultural factors in relation to plant disease is crop residue.

Crop succession is one strategy to reduce dependence on chemical fertilization, with emphasis on legumes, which provide more residual nitrogen and reduce the need for topdressing fertilization. Legume plants, besides symbiotic nitrogen fixation, present a higher rate of decomposition, with consequent release of nutrients, providing minerals for the subsequently cultivated crop (LOURENTE *et al*., 2007). However, this practice may increase the incidence of disease and consequent mycotoxin production, especially when host crops are used as predecessor to wheat (maize) cultivation (BLANDINO *et al*., 2012).

Inoculation with *Azospirillum* spp., a bacterium capable of fixing nitrogen (N_2) and producing hormones that stimulate plant growth (auxin and gibberellin), may result in significant variation in growth parameters in different cereals, including traits such as plant height, leaf size, number of tillers, root length, and root volume (MOREIRA *et al*., 2010). Research suggests that the inoculant does not replace nitrogen fertilizer but may promote better absorption and use of available nitrogen, increasing productivity as the crop is inoculated (VOGEL *et al*., 2013).

In addition to the challenges faced by the wheat crop, plant diseases also appear as a limiting factor, with emphasis on *Fusarium* Head Blight (FHB). After infection in the field, in addition to reducing plant productivity, the fungus (*Fusarium graminearum*) under stress conditions can produce secondary metabolites known as mycotoxins, which can function as insecticides, besides playing a role in fight against plant defense and assist the fungi in some way to compete for their ecological niche in nature (DE BOEVRE; GRANICZKOWSKA; SAEGER, 2015; MACHADO *et al*., 2017). Deoxynivalenol or vomitoxin (DON) is the predominant and most economically important toxin in the production and safety of kernels, even though DON is less toxic than other trichothecenes such as nivalenol (NIV) and sterol zearalenone (ZEA), also produced by *F. graminearum* (SOBROVA *et al*., 2010). Strategies to reduce mycotoxin contamination levels are linked to the control of pathogen and development of disease in the field, however the presence of disease is not synonymous of mycotoxin production (CARVALHO *et al*., 2013; TERZI *et al*., 2014). Blandino *et al*. (2012), analyzing kernels naturally contaminated by DON in field experiments, observed that the variables evaluated (controlled) presented the following order of iniquity in mycotoxin production: susceptibility of the cultivar ≥ predecessor crop ≥ planting system ≥ application of fungicide in the period of anthill of wheat.

Thus, we seek a better understanding of the effect that common agricultural practices have on the quality of kernels produced. Therefore, field trials were conducted aiming to evaluate the impact of agricultural management practices on the quality of wheat kernels, focusing on crop succession (maize or soybean), nitrogen dose applications in topdressing (0, 30, 60, 90, and 120 kg ha^{-1}), inoculation of seeds with *Azospirillum brasilense* and interactions of factors.

MATERIAL AND METHODS

Material and inputs

The total cycle (until harvest maturity) of cultivar BRS 220, as observed in variety trials conducted at experimental stations located in different adaptation regions of the State of Paraná (2000 to 2002), ranges from 103 to 128 days, with an average of 118 days (early to medium cycle). In addition, the cultivar presents the following characteristics: 265 10^{-4}J of dough strength (W), 3.2 of tenacity/extensibility ratio (P/L), and production of 4,853 kg ha^{-1}, belonging to the bread wheat class, according to the Brazilian wheat commercial classification, (BASSOI *et al*., 2005).

Prior to sowing, untreated seeds (insecticide and/or fungicide) were divided into two portions being one treated with liquid inoculant containing *Azospirillum brasilense* strain Ab-V5 and Ab-V6 at a concentration of 1×10^{8} viable cells mL^{-1} (seed inoculation factor), at the rate of 4 mL of the commercial product for each 1 kg of seed, two hours before sowing and using a polyethylene bag for homogenization (Azototal®, Total Biotechnology®, Curitiba, Brazil).

Characterization of experimental area

Field experiments were conducted during 2010 and 2011 crop seasons at Embrapa-CNPSo (Brazilian Agricultural Research Corporation - Soybean Center), located in the district of Warta, Londrina, Paraná State, Brazil. The District of Warta (Londrina) is located at 23º 11' S, 51º 10' W, with an average altitude of 605 m, and soil characterized as rhodic hapludox. The region, according to Köppen, is Cfa, or subtropical climate with the average temperature in the coldest month going below 18 °C (mesothermal), and the average temperature in the hottest month going above 22 °C; the climate is characterized by hot summers, infrequent frosts, and a tendency for rainfall concentration during the summer but no defined dry season. The average temperature data (°C) and daily rainfall (mm) during the crop cycles were provided by the meteorological station, located approximately 2 km from the experiment site (Figure 1).

The experimental area was managed under a no-tillage system, with wheat sowing carried out in succession to soybean or maize crop (crop succession factor). Prior to installation of the experiments, samples were collected from the first 20 cm of the soil layer for chemical analysis. The results obtained for the areas where soybean or maize was the predecessor crop, respectively, were: pH ($CaCl_2$): 4.87 and 5.09; C: 15.33 and 12.49 g dm^{-3}; P ($Mehlich^{-1}$): 8.41 and 6.85 mg dm^{-3}; H + Al: 4.95 and 5.27 $cmol_c$ dm^{-3}; K: 0.69 and 0.74 $cmol_c$ dm^{-3}; Ca+Mg: 6.05 and 7.07 $cmol_c$ dm^{-3}; Cation exchange capacity (CEC): 11.69 and 13.08 $cmol_c$ dm^{-3}; Base saturation (V): 58 and 60%; and clay content of 730 and 745 g kg^{-1}.

Mineral fertilization at sowing was performed with nitrogen, phosphorus, and potassium (NPK) based on soil analysis results, according to guidelines of the Brazilian Technical Commission Indications for Wheat and Triticale Research. For this, 250 kg ha^{-1} of the formula 8-28-16 was used, which corresponds to 20 kg ha^{-1} of N, 70 kg ha^{-1} of P_2O_5, and 40 kg ha^{-1} of K_2O. Ammonium nitrate was used as source of nitrogen (32% nitrogen and 3% K_2O) and applied in topdressing at the start of tillering (Stage 2 of Feekes Scale), according to the pre-established doses (nitrogen doses factor). The amount of K_2O was corrected and all parcels received the same amount of potassium.

Experimental design

Two independent field trials in the same location were conducted for the factor crop succession (maize or soybean) using a completely randomized block having a split plot design, with four replications. The seed inoculation factor was allocated in the main plot and nitrogen doses (0, 30, 60, 90, and 120 kg ha^{-1}) in the subplot. The experimental plot consisted of 13 rows, each 8 m in length at 17 cm spacings. The harvested or usable area for each experimental plot was constituted by the seven central rows (excluding 3 rows on each side), ignoring 1.25 m at the ends, totaling 6.54 m^2 of floor area (Figure 2).

Figure 1 - Climate data: daily average temperature (line) and precipitation (bar) during crop development. SE: Seeding, NI: Nitrogen fertilization, HE: Heading stage, HA: Harvest

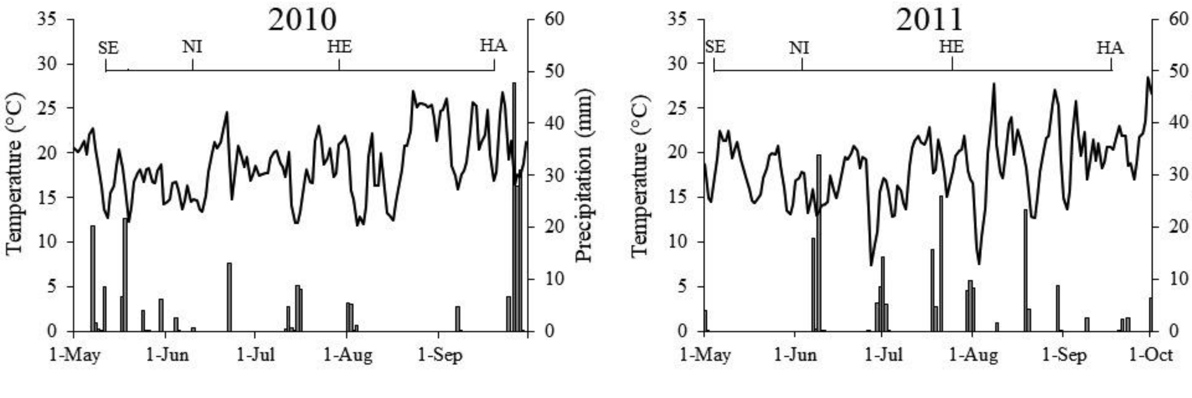
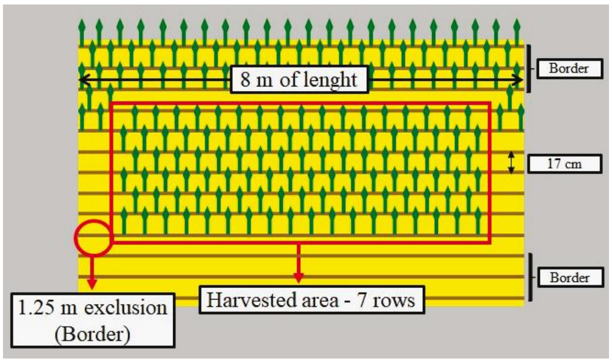


Figure 2 - Diagram of the experimental area



Operational procedure

A mechanical seeder-fertilizer machine for a no-tillage system was used in the experiment, obtaining an approximate plant density of 300 per m^2. Cultural practices were carried out according to the guidelines of the Brazilian Wheat and Triticale Research Commission (REUNIÃO DA COMISSÃO BRASILEIRA DE PESQUISA DE TRIGO E TRITICALE, 2010). The experimental area was monitored weekly and fungicide was applied at the onset of disease symptoms in 2010 (epoxyconazole 45 g ha^{-1} and pyraclostrobin 119.7 g ha^{-1} applied 59 days after emergency - DAE) and 2011 (tebuconazole 1 L ha^{-1} at 62 DAE). Weed control was performed when necessary if weeds were observed at a critical period for the crop (emergence to heading) in 2010 (bentazon 720 g ha^{-1} applied 19 DAE) and 2011 (glyphosate 3 L ha^{-1} applied in the area 7 days before sowing and metsulfuron-methyl 3 g ha^{-1} at 59 DAE). Insecticide was applied when insect pest infestation levels reached the limits of economic damage in 2010 (thiamethoxam 7.05 g ha^{-1} and lambda-cyhalothrin 5.3 g ha^{-1} applied 59 DAE) and 2011 (thiamethoxam 21.2 g ha^{-1} and lambda-cyhalothrin 15.9 g ha^{-1} applied 59 DAE).

Mechanical harvesting of the experimental plot occurred at stage 11.4, corresponding to kernels presenting moisture content lower than 20%. After cleaning, the sample was ground to 30 mesh in a hammer mill (MOD MA-090, Marconi®, Piracicaba-SP, Brazil), with cleaning of the equipment between two successive operations to avoid cross contamination (using 70% alcohol), and the ground samples were stored at -18 °C (MOD FE26, Electrolux®, Manaus, Brazil) until analysis.

Methods

Chemical composition

Chemical composition was analyzed on the samples obtained from milling the kernels to 30 mesh and the results are expressed on a dry basis. To measure the moisture content, samples were dried for 72 h at 103 ºC (MOD NV 1.5, Nevoni®, São Paulo, Brazil), as described by AACC International Approved Method 44-15.02 (AMERICAN ASSOCIATION OF CEREAL CHEMISTS INTERNATIONAL, 1999c). Protein content was measured by the Kjeldahl method (AACCI method 46-12.01d), using a block digester (MOD TE-40/25, Tecnal®, Piracicaba, Brazil) and a nitrogen distiller (MOD TE-036/1, Tecnal®), converting the total nitrogen content into protein, AACCI Approved Method 46-19.01 (AMERICAN ASSOCIATION OF CEREAL CHEMISTS INTERNATIONAL, 1999e). Lipid content (crude fat) was measured in pre-hydrolyzed samples (5 g sample/50 mL of 4M hydrochloric acid), using Soxhlet extractor refluxing 150-200 mL of ether petroleum (MOD TE-188, Tecnal®; MOD MA-487, Marconi®), as described in the AACCI approved method 30-25.01 (AMERICAN ASSOCIATION OF CEREAL CHEMISTS INTERNATIONAL, 1999b). Ash content was measured by weighing the muffled incineration residues (MOD 318D24®, Quimis®, Diadema, Brazil; AACCI method 08-01.01 (AMERICAN ASSOCIATION OF CEREAL CHEMISTS INTRNATIONAL, 1999a). Starch content was measured by enzymatic hydrolysis (Protocol PTF) and analysis in UV-visible measurements (MOD British S22, Biochrom®, Cambridge, United Kingdom), as described by Walter, Silva and Perdomo (2005). Total carbohydrate content was calculated as follows: total carbohydrate % = 100% - (protein % + lipids % + ash %). Analyses were carried out in duplicate or triplicate, if necessary.

Deoxynivalenol contamination

Deoxynivalenol-DON quantification was performed by the enzyme immunoassay ic-ELISA (Indirect Competitive Enzyme Linked Immunosorbent Assay) method as described by Santos *et al*. (2011). The absorbance was read (λ = 450 nm) in an ELISA reader (ASYS MOD Expert Plus Biochrom®, Cambridge, UK).

The limit of detection (LOD) and limit of quantification (LOQ) corresponds to 3- and 10-folds the standard deviation of the blank, respectively, calculated to a concentration through the calibration curve constructed from the average of 7 standard curves. The LOD obtained was 14 ng mL^{-1} (corresponding to 113 ug kg^{-1}) and the LOQ obtained was 56 ng mL^{-1} (corresponding to 445 ug kg^{-1}). Sample contamination was considered not detected (ND) when contamination was below LOD. The method showed an average recovery of 103% of DON (artificial contamination of 350, 750, and 1750 ug kg^{-1}) and average relative standard deviation (RSD) of 12.8%.

Statistical analysis

The exploratory analysis of the data was performed to verify the fulfillment of the assumptions for analysis of variance (normality and homoscedasticity), using SISVAR version 5.6, System for Analysis of Variance (FERREIRA, 2011). The data were grouped in a single analysis for crop succession, separately by crop year, since the ratio between the largest and the smallest mean squared error (MSE) was lower than 7. The effects of nitrogen doses were analyzed by regression test up to 2nd degree, at 5% probability. The means of crop succession (after maize or soybean) and inoculation of seeds with *Azospirillum brasilense* were submitted to test F at 5% of significance.

RESULTS AND DISCUSSION

Immobilization or lower availability of nitrogen provided by the degradation of predecessor crop residue (soybean or maize) into the soil can be observed in Figure 3, which shows only the significant results obtained in the analysis of variance ($p<0.05$).

As expected, nitrogen fertilization showed a more pronounced effect under maize succession when compared to soybean succession, since the degradation of soybean residue is a good source of nitrogen for the plants. This effect is evidenced in 2011 once the protein content in the wheat kernels grown after soybeans crop was 27.5% higher than in the wheat kernels grown after maize, when no nitrogen was applied in topdressing (0 kg ha^{-1}; Figure 3E), i.e., having only the predecessor crop residue as nitrogen source. The results obtained were as expected, since wheat's response to the application of mineral nitrogen is dependent on the predecessor crop (PINNOW *et al*., 2013). Protein content did not differ among soybean/wheat and maize/wheat treatments when applying high doses of nitrogen (120 kg ha^{-1}), once there was no competition for the nutrient (Figure 3E). However, different results were observed in the first year evaluated.

The differences observed when low doses of nitrogen were applied (≤90 kg ha^{-1}) is due to availability of nutrients provided by each culture (maize or soybean), which were incorporated into the soil and subsequently used by the wheat plants. The C/N ratio of maize crop residues is higher, promoting greater immobilization of N in the soil, reducing its availability to the succeeding crop (REGEHR *et al*., 2015).

Application of increasing doses of nitrogen in succession to maize led to a higher increase in protein content in the kernels (+48.25%; 0-120 kg ha^{-1}), with a positive linear correlation between the variables ($y=0.05x+11.45$; $r^2=0.979$; $p<0.01$; Figure 3E). In succession to soybean, the protein content increased by 16.28% (0-120 kg ha^{-1}), with a positive linear correlation between nitrogen doses and protein content ($y=0.02x +14.57$; $r^2=0.882$; $p<0.01$; Figure 3E).

These results are important as the technological quality of the flour and its suitability for industry are mainly determined by protein content and its variation, qualitatively in terms of subunit composition and, quantitatively, in terms of the amount of different protein fractions that form the gluten (FRANCESCHI *et al*., 2009).

On the contrary, according to the results shown in Figure 3G, the greater the availability of nitrogen to the plant, the smaller the starch content in the kernel produced. Wheat cultivated after maize (0 kg ha^{-1}) had 6.58% higher starch content in the kernel than when grown after soybean, which provides more nitrogen to the plant after decomposition, as well as under conditions of high rates of nitrogen in topdressing (120 kg ha^{-1}; Figure 3G).

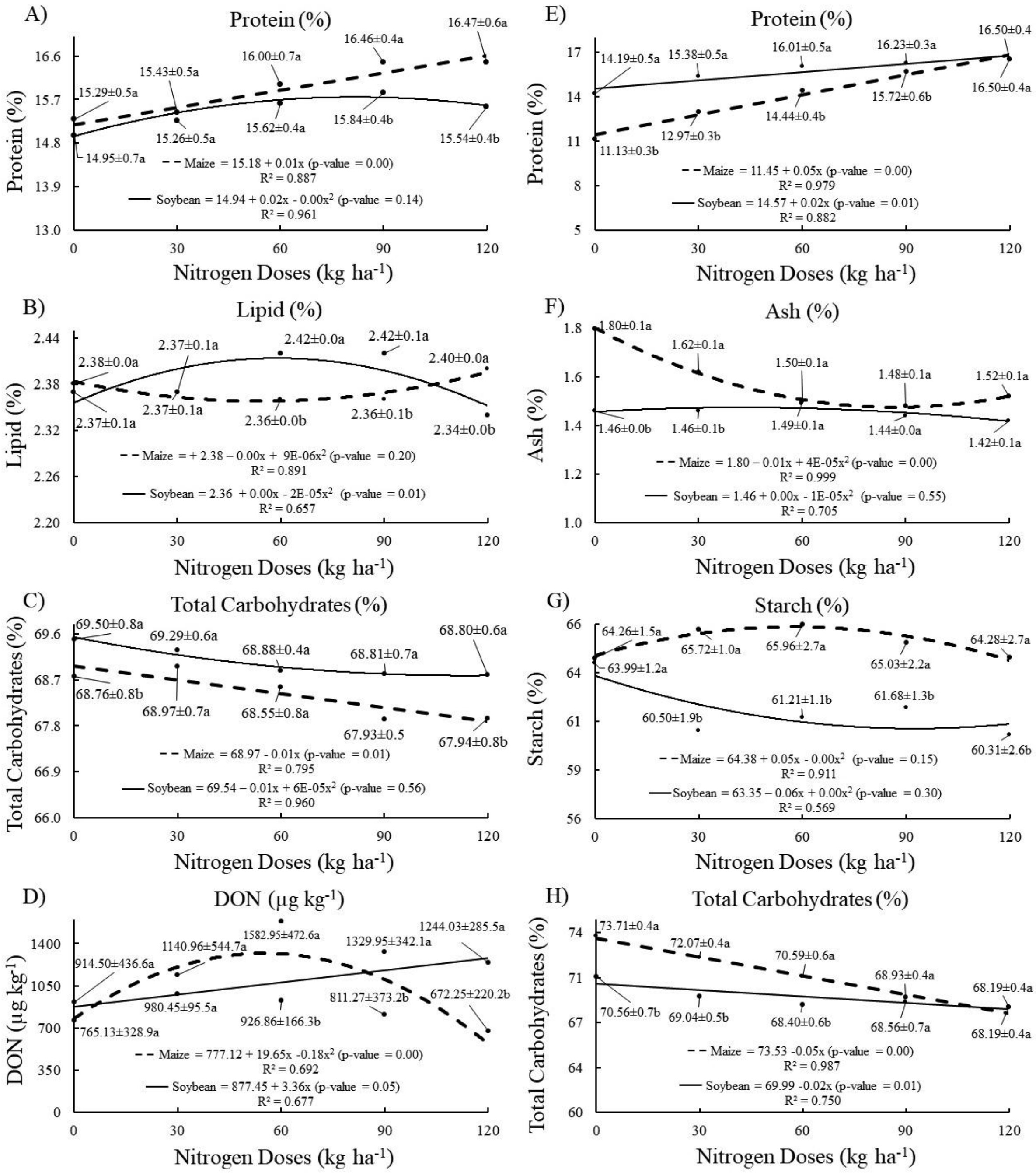
Starch and total carbohydrate content in response to nitrogen fertilization were inversely related to protein content, presenting a negative correlation (Starch - $r = -0.37$; Total carbohydrates- $r = -0.99$; $p<0.01$). Nitrogen is the main protein-forming component. Thus, under conditions of low nitrogen availability, the plants decrease protein synthesis in the kernels and favor starch synthesis (PINNOW *et al*., 2013). Studies have shown that nitrogen deficiency condition usually result in accumulation of nonstructural carbohydrates in the kernels (KOVACEVIC *et al*., 2012). Nitrogen deficiency can lead to sugar accumulation, reducing the use of carbon skeletons for synthesis of amino acids and proteins (WINGLER *et al*., 2006).

According to the guidelines of Brazilian Wheat and Triticale Research Commission for Paraná State, the amount of nitrogen fertilizer applied in topdressing at tillering stage is dependent on the crop residue present in the soil (soybean – 30 to 60 kg ha^{-1}; maize – 30 to 90 kg ha^{-1}). Thus, the doses evaluated in this study (0, 30, 60, 90, and 120 kg ha^{-1}) aimed to simulate conditions of excess and deficiency of nitrogen supplied to the plants through topdressing fertilization (REUNIÃO DA COMISSÃO BRASILEIRA DE PESQUISA DE TRIGO E TRITICALE, 2010).

These results, together with those observed for protein content, reflect the results observed for total carbohydrate content in the kernels, which is consist essentially of starch (Figure 3H).

The negative effect of reducing starch content is that, depending on the end use of flour, one component of flour may be more important than the others. In products such as cake batter, there is no significant formation of gluten network and the main component of cake structure formation is starch (CAUVAIN, 2017). For bread production, quality and quantity of proteins are important, as proteins are responsible for gluten formation, the major and most crucial component of dough, which is linked directly with bread quality (MARCHETTI *et al*., 2012).

Figure 3 - Chemical composition and deoxynivalenol contamination of wheat kernels (*Triticum aestivum* L.; BRS 220) in response to interaction of factors (crop succession × nitrogen doses), in the 2010 (A-D) and 2011 crop year (E-H). Mean ± standard deviation (dry basis); DON = Deoxynivalenol; Means followed by different lower-case letters indicate differences between trend lines (maize or soybean), by Tukey's test at 5% probability



Significant differences for DON contamination in the kernels were observed between maize or soybean as a predecessor crop when high doses of nitrogen were applied in topdressing (>60 kg ha^{-1}; p>0.05; Figure 3D). In general, the contamination observed was higher when wheat was cultivated after soybean, despite maize residue serving as a potential source of inoculum. However, it is known that fungal growth and toxin production are not synonymous, e.g., the best growth condition for fungus do not always coincide with toxin synthesis (MARROQUÍN-CARDONA *et al*., 2014).

Blandino *et al*. (2012) observed that the main factors in the production of DON in wheat grain follow this order: susceptibility of the cultivar ≥ predecessor crop ≥ planting system ≥ period of fungicide application. According to the authors, the use of host crop as predecessor, especially maize and sorghum, which increase the amount of inoculum in the field, and the use of susceptible cultivars, contribute to the maximal contamination of wheat crop by *Fusarium*.

The significant results for the interaction between inoculation of seeds with *A. brasilense* (inoculated or control) and application of increasing doses of nitrogen in the topdressing (0-120 kg ha^{-1}) are shown in Figure 4 (p<0.05).

For all the variables evaluated in the kernels (chemical composition and contamination by deoxynivalenol), only ash/mineral content seems to have suffered a significant effect when the seeds were inoculated with *A. brasilense*. As shown in Figure 4G, the application of nitrogen doses higher than 30 kg ha^{-1}, in plants obtained from seeds inoculated with *A. brasilense*, reduced the ash content found in the kernel (-10.9% when applying 120 kg ha^{-1}).

Piccinin *et al*. (2013), using the same strains (Ab-V5 and Ab-V6) and concentration of inoculum (108 UFC mL^{-1}) in the years 2010 and 2011 in Maringa, PR, concluded that *A. brasilense* is an alternative to partially meet the plant's demand for nitrogen, with supplementation by nitrogen fertilizer. The authors observed that applying half the recommended rate of nitrogen along with the inoculant provides positive results in agronomic performance and wheat productivity. Inoculation of wheat plants does not replace nitrogen fertilizer, but it promotes better absorption and utilization of nitrogen available for increased root growth (MOREIRA *et al*., 2010; VOGEL *et al*., 2013).

Pinheiro *et al*. (2002), observing the influence of different factors on the absorption of 10 different strains of *Azospirillum* isolated from the roots and rhizosphere of wheat, found that, with one exception (*A. brasilense* - SpBr14), the optimum absorption of bacteria in the roots occurs at pH 6.0. Thus, the high soil acidity (pH 4.9) in the experimental area may have reduced the adsorption of bacteria and the availability of molybdenum, a constituent of nitrogenase, responsible for nitrogen fixation (HOFFMAN *et al*., 2014). Besides, acidic soil constitutes a negative factor for the plant, due to the possible reduction of the availability of minerals at pH<5, particularly nitrogen, phosphorus, calcium, and magnesium (GOULDING, 2016).

Despite the differences observed for ash (mineral) and lipid content in the kernels (Figure 3 and 4) in response to the variables evaluated in this study (crop succession, inoculation of seeds with *A. brasilense* and nitrogen doses), the changes would not have substantial effect on the usability of the flour obtained, since protein and starch quantity and quality are the attributes that most influence the technological quality of wheat (FRANCESCHI *et al*., 2009).

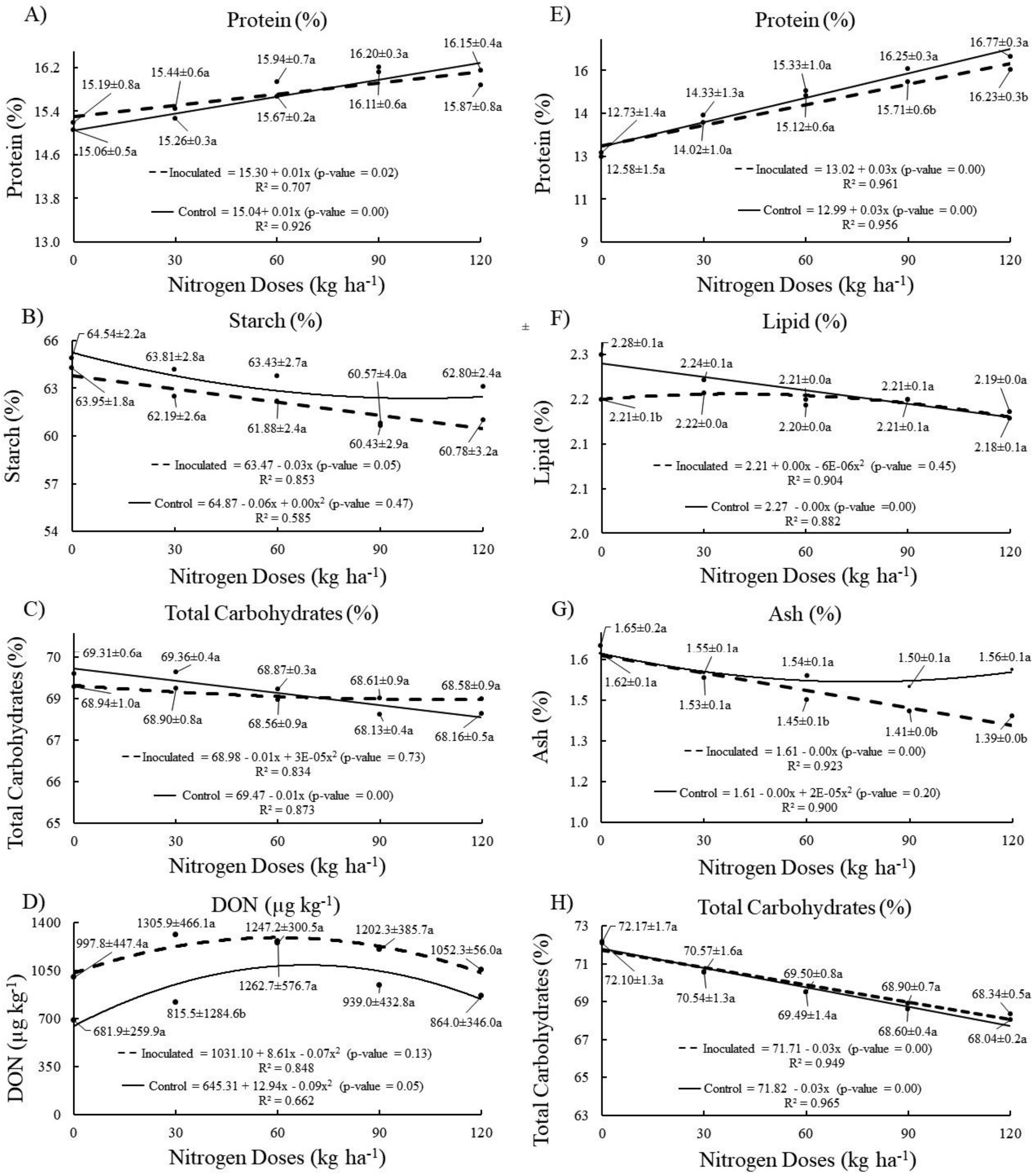
In terms of safety of whole wheat flour obtained from the kernels produced here, although constant rain and optimum temperature for growth of *Fusarium graminearum* were observed in the second year (Figure 1), higher levels of DON contamination were observed in the first year of evaluation (Figure 5). The water deficit observed in the first year may have contributed to plant weakness, reducing its resistance to disease, and created a stress condition to fungi growth which stimulated the production of mycotoxin (BLANDINO *et al*., 2012; MARROQUÍN-CARDONA *et al*., 2014).

Contamination in the first year ranged from 247.6 (<LOQ) to 2355.9 ug kg^{-1}, and in the second year from ND (<LOD) to 1474.0 ug kg^{-1}. Therefore, 68.8% (55/80) and 97.5% (78/80) of the samples from the first and second year (Figure 5), respectively, showed contamination by DON below the maximum tolerated limit for whole wheat flour (<1250 ug kg^{-1}), as established by Resolution - RDC no. 138 (BRAZIL, 2017).

However, according to the Resolution, starting on 1st January 2019, the acceptable level of DON contamination in whole wheat flour will be lower (<1000 ug kg^{-1}). Thus, considering the new maximum tolerated limit, 60% (48/80) and 88.8% (71/80) of the samples from the first and second year, respectively, would display contamination below the limit (Figure 5).

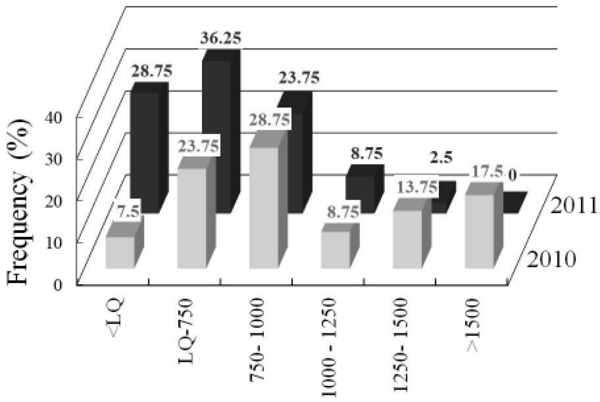
According to Terzi *et al*. (2014), reducing the level of cereal head infection caused by *Fusarium*, and associated mycotoxin accumulation in grains, is a high priority in order to secure the yield, agronomic performance, and food and feed safety from field to table.

Figure 4 - Chemical composition and deoxynivalenol contamination of wheat kernels (*Triticum aestivum* L.; BRS 220) in response to interaction of factors (*Azospirillum brasilense* × nitrogen doses), in 2010 (A-D) and 2011 (E-H). Mean ± standard deviation (dry basis); DON = Deoxynivalenol; Means followed by different lower-case letters indicate differences between trend lines (inoculated or control), by Tukey's test at 5% probability



Crop residues may have experienced reduced degradation due to low rainfall in occurred in the first crop year (95.9 mm), which was only half the amount of rainfall occurred in the second year and provided only one third the amount of water required by the plants, causing the inoculum of *F. graminearum* to remain viable in the soil for a longer period. In addition, according to Marroquín-Cardona *et al*. (2014), water deficit contributed to plant weakness, reducing its resistance to disease (Figure 1). As known, temperature and humidity are important factors

Figure 5 - Distribution (%) of deoxynivalenol-DON contamination levels (ug kg^{-1}) in samples (n =160) of wheat (*Triticum aestivum* L.; BRS 220) from the southern region of Brazil, north of Parana State



in the degradation of plant debris, since they determine the growth rate of microorganisms, and therefore, the decomposition of the residue (VILLAR *et al*., 2016).

CONCLUSIONS

1. Crop succession (maize or soybean) and application of increasing doses of nitrogen fertilizers (0, 30, 60, 90 e 120 kg ha^{-1}) changed the chemical composition of wheat kernels produced (BRS 220), which can influence its end use;
2. Cultivation of wheat after maize crop, without application of nitrogen fertilizer in topdressing (0 kg ha^{-1}), produced kernels with lower protein (-21.6%) and higher carbohydrate content (+4.5%) than those cultivated in succession to soybean crop. However, application of large amounts of nitrogen (120 kg ha^{-1}) canceled the residue effect (maize or soybean) as the fertilizer provided all the nitrogen required by the plants;
3. Owing to climatic adversities observed during crop development (drought), further studies are needed to evaluate whether seed inoculation with *Azospirillum brasilense* could be recommended as a strategy to increase the quality and safety of kernels.

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