Date: Dec 18, 2021
To: "Agustín Francisco Arata" arataa@faa.unicen.edu.ar
From: "Journal of Food Composition and Analysis" support@elsevier.com
Subject: Decision on submission to Journal of Food Composition and Analysis

Manuscript Number: JFCA-D-21-00831R2

Effects of sowing date, nitrogen fertilization, and Fusarium graminearum in an Argentinean bread wheat: integrated analysis of disease parameters, mycotoxin contamination, grain quality, and seed deterioration.

Dear M.Sc. Arata,

Thank you for submitting your manuscript to Journal of Food Composition and Analysis.

I am pleased to inform you that your manuscript has been accepted for publication.

My comments, and any reviewer comments, are below.
Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Journal of Food Composition and Analysis and hope you will consider us again for future submissions.

Kind regards,
Percy Onianwa
Handling Editor

Journal of Food Composition and Analysis

Editor and Reviewer comments:

More information and support

FAQ: When and how will I receive the proofs of my article?
https://service.elsevier.com/app/answers/detail/a_id/6007/p/10592

You will find information relevant for you as an author on Elsevier's Author Hub: https://www.elsevier.com/authors

FAQ: How can I reset a forgotten password?
https://service.elsevier.com/app/answers/detail/a_id/28452/support

For further assistance, please visit our customer service site: https://service.elsevier.com/app/home/supporthub/publishing/. Here you can search for solutions on a range of topics, find answers to frequently asked questions, and learn more about Editorial Manager via interactive tutorials. You can also talk 24/7 to our customer support team by phone and 24/7 by live chat and email.

#AU_JFCA#

To ensure this email reaches the intended recipient, please do not delete the above code

---

Journal of Food Composition and Analysis

Effects of sowing date, nitrogen fertilization, and Fusarium graminearum in an Argentinean bread wheat: integrated analysis of disease parameters, mycotoxin contamination, grain quality, and seed deterioration.

--Manuscript Draft--

Manuscript Number:	JFCA-D-21-00831R2
Article Type:	Research Paper
Section/Category:	
Keywords:	Fusarium graminearum; Food analysis; Food composition; gluten; mycotoxin; nitrogen; seed viability; sowing date
Corresponding Author:	Agustín Francisco Arata Azul, Buenos Aires ARGENTINA
First Author:	Gonzalo J. Arata
Order of Authors:	Gonzalo J. Arata
	Mauro Martínez
	Constanza Elguezábal
	Dante Rojas
	Diego Cristos
	María Inés Dinolfo
	Agustín Francisco Arata
Abstract:	Bread wheat is one of the main crops cultivated worldwide that can be affected by fungal diseases as Fusarium head blight. The aim of this work was to analyze the effect of sowing date and nitrogen fertilization on the F. graminearum -wheat interaction. The experiment was carried out involving two sowing dates with different nitrogen fertilization and F. graminearum inoculation treatments. A total of 32 variables related to the disease parameters, mycotoxin production, grain quality, gluten composition, and seed deterioration were evaluated. Disease severity was significantly increased after F. graminearum inoculation (2.2-fold) while mycotoxin production increased significantly for the delayed sowing date: deoxynivalenol (3.2-fold), 3-ADON (3.6-fold), nivalenol (8-fold), and zearalenone (5-fold). Regarding grain quality, the delayed sowing date showed a decrease in the protein percentage (-15%) and gluten concentration (-17.9%), while an increase of test weight (+6.2%) were observed. Seed deterioration variables showed differences among longevity parameters showing an increase in seed vigor and viability in the late sowing date. In conclusion, sowing date and nitrogen fertilization could affect FHB severity, mycotoxin contamination, and grain quality.
Response to Reviewers:	

Azul, 26 of November 2021

Dear Percy Onianwa

Editor-in-chief

Journal of Food Composition and Analysis

I am sending the new version of the manuscript titled "**Effects of sowing date, nitrogen fertilization, and *Fusarium graminearum* in an Argentinean bread wheat: integrated analysis of disease parameters, mycotoxin contamination, grain quality, and seed deterioration".** We hope that this new version is now suitable for publication in *Journal of Food Composition and Analysis.*

Best regards.

Agustin Arata

Azul, 26 of November 2021

Dear Percy Onianwa

Editor-in-chief

Journal of Food Composition and Analysis

I am sending the new version of the manuscript titled "**Effects of sowing date, nitrogen fertilization, and *Fusarium graminearum* in an Argentinean bread wheat: integrated analysis of disease parameters, mycotoxin contamination, grain quality, and seed deterioration".** We hope that this new version is now suitable for publication in *Journal of Food Composition and Analysis.*

Best regards.

Agustin Arata

Editor's Comment:

1) As far as possible, the abstract should contain some real analysis data (of concentrations) from the study.

Real analysis results were included in Abstract section. (Page 1, lines 12-24)

2) Keywords for manuscripts in this journal should include "food analysis" and "food composition".

The word recommended have been added (Page 2, Line 26-27)

3) Extensive use of abbreviations in the manuscript is undesirable and make the work difficult to easily read. This should be minimised. In fact, no single word, or even two words term, should be represented with an abbreviation!

Some abbreviation words were removed from Abbreviation section and modified along the manuscript included in Figures and Tables.

4) Cited procedures need to be fully described! E.g. lines 260-261

The description procedures were fully described (Pages 11-12, lines 272-284)

5) Vendors details, using the appropriate format (vendor name, city, country) must be provided for all equipment, chemicals, standards, etc, in the Materials and methods section. Several of your vendors' details do not adhere to this format. Vendors details were even not provided for some equipment and software.

Vendor details were added along Material and Method section.

6) The preference of the journal is for the Results and Discussion to be a single section. Revise accordingly!

Results and Discussion were both described in a single section according to the editor's suggestion.

7) Table titles are too lengthy and not acceptable. Any explanation of terms or abbreviations in the tables should be presented as footnotes to such tables, and not within the titles.

Table tiles were modified.

8) The reference citation and listing style in the manuscript does not adhere to the prescribed format for the JFCA. Authors should consult the JFCA Authors guideline. For example, the names of journal should be written in full and not abbreviated.

The reference section was modified according to the reviewer's suggestions and Authors guidelines (Pages 27-36, lines 654-869).

9) Overall, this journal is a food composition/nutrition journal and not a strictly agronomic journal. Emphasis in its published papers should tilt towards compositions, nutrition and methodology. This paper needs to emphasise more in this regard.

References were added to highlight the importance of mycotoxins in food change and the relevance of nutrition in grain composition (Flaete et al. 2005; Palacios et al. 2017; Tóth et al. 2019).

10) The revisions should be carried out using Track Changes so that editors and reviewers can easily verify that appropriate changes have been made. The revised manuscript should be line numbered. Manuscripts that are revised without track changes may not be processed, and may be returned to authors.

The entire manuscript was modified using Track Changes, according to the reviewer's suggestions.

11) When responding to reviewers and editor's comments, any changes made must be pointed out by referring to appropriate line number positions in the revised version.

Revised manuscripts may be returned to authors without further processing by the editor if authors do not comply.

The modifications performed in the revised manuscript were referred to the appropriate line number (see Response to the editor).

Reviewer #1: The paper describes a study on integration of the data from complex experiment involving N fertilization, *Fusarium graminearum* infection and sowing date alterations, conveyed to reveal the relationship between these traits and their impact on the FHB severity and mycotoxin production.
The manuscript was generally well-prepared and presents some new results, however, it is overwhelmed with data and could be more compact. Some parts of it, including the information given need careful attention and improvement. My main specific comments are pointed out below.

Introduction is chaotic at times (first F. graminearum and then Fusarium in general - lines 101-106) and contains some information that belong to the Discussion rather than here (e.g. lines 122-132).

Introduction section was improved according to the reviewer's suggestions. (Page 4-7, lines 95-163).

Materials and methods' description is exhaustive and gives all the information needed for repeating the experiments. I have no specific comments for this section.
Results: the description of the results is focused, but does not read well, it is really hard to follow the story with all these data (e.g. paragraphs in lines 310-326). I would suggest to extract the most important data from all obtained and focus on them, leaving all the remaining ones in the tables. I also miss the solid data on mycotoxin contamination, i.e. actual concentrations of individual metabolites for each experimental variant. This would be much more informative for the readers.
Discussion is a strong point of this article. The cited references are appropriate and conclusions are well supported by the results.

Result and discussion sections were modified according to the editor's comments and were grouped in a unique section.

Reviewer #2: Dear Authors. The submitted manuscript concerns an important problem in both agronomic and nutritional aspects. After reviewing the manuscript, I conclude that it requires major revision before possible publication. All comments can be found in the attached pdf file posted as yellow "Comments" (21 comments in total), so I no detail my remarks here.

One comment is most important, and the Authors need to address this in the revised version of the manuscript. Only one wheat cultivar, Klein Proteo, was examined. Bread wheat is a self-pollinating plant, so the statements about the response of a crop species (wheat) based on the reaction of a single homozygous genotype (Klein Proteo) is

incorrect. For this reason, the title of the manuscript needs to be changed and those fragments where a cultivar is identified with the species (wheat) must be necessarily rewritten.

C1: The title of the manuscript was modified (Page 1, lines 1-3).

C2: Conclusion in the abstract section was rewritten (Page 1, lines 21-24).

C3: FDK abbreviation was corrected (Page 2, line 39).

C4: GLI/GLU abbreviation was removed (Page 2, line 42).

C5: HMW/LMW abbreviation was removed (Page 3, line 57).

C6: N abbreviation was removed (Page 3, line 64).

C7: The susceptibility of cv. Proteo was clarified (Page 8, lines 178-181).

C8: ´L´ was replaced by ´l´ along the manuscript.

C9: LOD and LOQ of ZEA were added (Page 11, lines 256-258).

C10: This statement unambiguously suggests that the artificial inoculation had no effect on the concentration of mycotoxins. How can this phenomenon be explained? Were the *F. graminearum* isolates examined for toxin productivity before experiment?

The *in vitro* mycotoxin production of the *F. graminearum* isolates has been evaluated in Castañares et al. (2014). Based on the results, these four isolates (3.4, 88.1, 92.2, and 129.1) were selected as inoculum. This information was added in the manuscript (Page 8, line 200).

The FHB disease parameters were affected by the inoculation treatment; however, this treatment did not affect the mycotoxin contamination. The high *Fusarium graminearum* inoculum pressure could explain these results in the field assay. Moreover, fungal growth and mycotoxin production do not require the same environmental conditions. Several works showed that while some conditions are optimal for fungal growth, these same conditions are not optimal for mycotoxin production. In conclusion, there is not a positive correlation between the fungal presence and mycotoxin production. For example, in Ramirez et al. (2006) the optimal conditions for *F. graminearum* growth and mycotoxin production are not the same (*International Journal of Food Microbiology* 106: 291-296).

C11: Slightly was removed along the manuscript.

C12: The groups were removed (Page 19, lines 451-459).

C13: Gorczyca et al. (2018) was clarified. Some sentences were modified (Page 17, lines 417-420).

C14: The sentence was modified (Page 17, line 414-417).

C15: A possible explanation has been answered in C10.

C16: The value of FDK in SD was written in bold letters.

C17: Was a (positive?) correlation between the SDSS and G?

The Pearson correlation between SDSS and G has been previously made. The p-value was 0.9453 showing no correlation among these variables.

Reviewer #3: The aim of this work was to analyze the effect of sowing date and nitrogen fertilization on the *F. graminearum*-wheat interaction. A lot of different analysis have been performed on the harvested grains. *F. graminearum* infection, fertilization, and weather conditions may all have an impact on grain quality. The interaction between *F. graminearum* and wheat is strongly dependent on weather conditions. Thus, it is difficult to conclude on the effects of sowing date and nitrogen fertilization on the *F. graminearum*-wheat interaction based on one field trial, only. The different weather conditions during flowering (and from flowering onwards) for the two sowing dates might have differently influenced the survival and infection by *F. graminearum*, in addition to grain quality. Many of the significant differences in the observed grain quality parameters were caused by sowing date, which makes it difficult to identify specific factors that have been causing this as the weather conditions the plants are exposed to at different developmental stages (such as flowering and grain filling) differs with sowing dates. If you wanted to identify the "effect of sowing date and nitrogen fertilization on the *F. graminearum*-wheat interaction" you could have reflected more on what different weather conditions that might be common for the various time periods (if any). Perhaps included a more detailed analysis of the weather parameters, especially around the time point of wheat flowering and onwards, and performed more than one field experiment. I do not understand why you have chosen to study the effect of 0 kg N/ha. I guess this is not a relevant fertilization treatment in Argentina? I think it would have been better to select a more appropriate and relevant fertilization treatment as the low N control. However, the small or often nonsignificant effect of N treatment on grain quality was somewhat surprizing.

I do find the topic interesting, however I would prefer that more than one field experiment was performed.

Weather conditions between both sowing dates have been described in the material and methods section, and these differences were used to discuss the results.

According to the level of N used, although we know that is not a dose used in our country, we try to test contrasting N doses, which explain the use of 0 kg N/ha under field conditions.

We agree that this kind of study should be repeated for almost two years. The authors argue that the amount of data generated in the present study is solid. Furthermore, due to the extensive number of variables measured, it would have been unnecessary to repeat it during another growing season for this purpose. Previous research conducted by the authors involving two or three years under field conditions indicates *Year* interaction. For this reason, and considering all the variables analyzed, we support that repeating this assay would be complex to discuss the results and reach solid conclusions. However, we will select those variables most

representatives for future works considering the repetition among different growing seasons.

Highlights

- Sowing date modifies ~~N~~nitrogen fertilization x *Fusarium graminearum* interaction.
- ~~FHB~~Fusarium head blight development can be affected by increasing ~~N~~nitrogen rates.
- Early sowing date shows better values of some grain quality parameters.
- Delay in sowing date would be related to high mycotoxin contamination.

Effects of sowing date, and nitrogen fertilization, and in *Fusarium graminearum* in an Argentinean bread wheat interaction: integrated analysis of disease parameters, mycotoxin contamination, grain quality, and seed deterioration.

Gonzalo J. Arata[a,b*], Mauro Martínez[c*], Constanza Elguezábal[d],

Dante Rojas[e], Diego Cristos[e], María I. Dinolfo[c], Agustín F. Arata[c,f]

[a] Instituto de Fisiología y Ecología Vinculado a la Agricultura, Consejo Nacional de Investigaciones Científicas y Técnicas (IFEVA-CONICET), Av. San Martín 4453, C1417DSE, Ciudad de Buenos Aires, Argentina.

[b] Cátedra de Cultivos Industriales, Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina. IFEVA, CONICET, Buenos Aires, Argentina.

[c] Laboratorio de Biología Funcional y Biotecnología (BIOLAB)-CICBA-INBIOTEC-CONICET, Facultad de Agronomía, UNCPBA. Cátedra de Mejoramiento Genético Vegetal, Facultad de Agronomía, UNCPBA. Av. República de Italia 780, Azul (7300), Buenos Aires, Argentina.

[d] Facultad de Agronomía, UNCPBA. Av. República de Italia 780, Azul (7300), Buenos Aires, Argentina.

[e] Área de Protección de Alimentos Instituto Tecnología de Alimentos Centro de Investigación de Agroindustria (CIA-INTA). Nicolás Repetto y de los Reseros s/n. 1686 Hurlingham, Buenos Aires, Argentina.

[f] Centro de Investigaciones Integradas sobre Sistemas Agronómicos Sustentables (CIISAS), Facultad de Agronomía, UNCPBA. Av. República de Italia 780, Azul (7300), Buenos Aires, Argentina.

Corresponding author. Email: arataa@faa.unicen.edu.ar, Tel./Fax: +54 (02281) 433291.

*Both authors contributed equally to this work.

Effects of sowing date, ~~and~~ nitrogen fertilization, and ~~in~~ *Fusarium graminearum* ~~-~~ in an Argentinean bread wheat ~~interaction~~: integrated analysis of disease parameters, mycotoxin contamination, grain quality, and seed deterioration.

Formatted: Font: Not Italic

Abstract

Bread wheat is one of the main crops cultivated worldwide that can be affected by fungal diseases as Fusarium head blight. The aim of this work was to analyze the effect of sowing date and nitrogen fertilization on the *F. graminearum*-wheat interaction. The experiment was carried out involving two sowing dates with different nitrogen fertilization and *F. graminearum* inoculation treatments. A total of 32 variables related to the disease parameters, mycotoxin production, grain quality, gluten composition, and seed deterioration were evaluated. Disease ~~parameters~~ severity ~~were~~ was significantly increased after *F. graminearum* inoculation (2.2-fold)~~,~~ while mycotoxin production increased significantly for the delayed sowing date: deoxynivalenol (3.2-fold), 3-ADON (3.6-fold), nivalenol (8-fold), and zearalenone (5-fold~~,~~).

Regarding grain quality, the ~~early~~ delayed sowing date showed a ~~better~~ decrease in the protein percentage (-15%) and gluten concentration (-17.9%), while~~,~~ an increase of ~~the late sowing date showed a better grain structure and~~ test weight (+6.2%) were observed. Seed deterioration variables ~~demonstrated slight~~showed differences among longevity parameters showing an increase in seed vigor and viability in the late sowing date. In conclusion, sowing date and nitrogen fertilization could affect FHB severity, mycotoxin contamination, and grain quality. ~~agronomic practices may influence the biotic and abiotic interaction, suggesting the importance of an integrated analysis to optimize the innocuity and quality of wheat grains.~~

Keywords *Fusarium graminearum*, food analysis, food composition, gluten, mycotoxin, nitrogen, seed viability, sowing date.

Abbreviations

DISK: deoxynivalenol content, incidence, severity, and kernel damage index

DON: deoxynivalenol

EC: electrical conductivity

EC0: electrical conductivity at the beginning of the accelerated aging assay

EC10: electrical conductivity at ten days of the accelerated aging assay

F: Fusarium graminearum treatment

F0: control treatment

F1: inoculated treatment

FDK: ~~*incidence and severity index*~~*Fusarium* damaged kernel

FHB: Fusarium head blight

~~G%: gluten percentage~~

~~GLI/GLU: ratio between contents of gliadins and glutenins~~

GLI: gliadins

GLU: glutenins

Glu-A1x/HMW: contents of glutenin subunit encoded by Glu-A1x locus relative to total high molecular weight glutenins

Glu-B1x/HMW: contents of glutenin subunit encoded by Glu-B1x locus relative to total high molecular weight glutenins

Glu-B1y/HMW: contents of glutenin subunit encoded by Glu-B1y locus relative to total high molecular weight glutenins

Formatted: Font: Italic

Glu-D1x/HMW: contents of glutenin subunit encoded by Glu-D1x locus relative to total high molecular weight glutenins

Glu-D1y/HMW: contents of glutenin subunit encoded by Glu-D1y locus relative to total high molecular weight glutenins

~~GR: global radiation~~

~~GW: grain weight~~

~~HMW/LMW: ratio between contents of high molecular weight and low molecular weight glutenin subunits~~

HMW: high molecular weight glutenin subunits

IPAR: intercepted photosynthetically active radiation

ISK: kernel damage index

LARC: lactic acid 5% v/v retention capacity

LMW: low molecular weight glutenin subunits

~~N: nitrogen~~

N0: unfertilized treatment

N1: fertilized treatment

NIV: nivalenol

~~Pp: precipitations~~

~~Pro%: protein percentage~~

~~RH: relative humidity~~

~~S.V.: source of variation~~

SCRC: sodium carbonate 5%w/v retention capacity

SD: sowing date

SD1: early sowing date

SD2: late sowing date

SDSS: sodium dodecyl sulfate sedimentation test

SPAD: estimated chlorophyll content in flag leaf

SRC: solvent retention capacity

SS%: percentage of the sum of squares respect to the model

SuRC: sucrose 50%w/v retention capacity

T50: time to reach 50 % of total germination

Tmax: maximum temperature

Tmed: mean temperature

Tmin: minimum temperature

~~TW: test weight~~

~~WC: water content~~

WRC: distilled water retention capacity

ZEA: zearalenone

3-ADON and 15-ADON: deoxynivalenol acetylated derivatives

α-β-γ-gli: α-β-γ-gliadins

ω-gli/α-β-γ-gli: ratio between ω-gliadins and α-β-γ-gliadins

ω-gli: ω-gliadins

1. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the main crops cultivated worldwide, as it is an important food source for direct consumption. The primary use of bread wheat is for flour production and the baking industry. The viscoelastic properties of wheat dough make it suitable for several bakery products, where gluten proteins play a significant role in determining wheat baking quality (Horvat et al., 2015). In this context, Fusarium head blight (FHB) is one of the most important fungal diseases that can severely affect crop

production ~~over the world~~worldwide. This fungal disease is observed mainly in regions with warm and wet climates during the flowering stage of several cereal crops (Reis & Carmona, 2002). In bread wheat, reductions in grain yield, grain quality, and seed viability, with the associated production of high mycotoxin concentrations, have been reported as the main adverse effects of FHB (Dubin et al., 1997). The type and amount of mycotoxins produced by *Fusarium* species can fluctuate among harvest seasons, depending mainly on climatic conditions, agronomic management, and storage conditions (Placinta et al., 1999). Trichothecenes are one of the most important mycotoxins produced by *Fusarium* spp. and have been frequently associated with feed refusal, vomiting, and suppressed immune functions in humans and animals (Desjardins, 2006). *Fusarium graminearum* sensu stricto, belonging to *Fusarium graminearum* species complex, is the prevalent species isolated worldwide, showing high variability in their genomic pool and toxigenic production (Ortega et al., 2016). ~~Regarding *F. graminearum*, t~~This pathogen ~~has the capacity to~~can produce a wide range of mycotoxins, mainly deoxynivalenol (DON) and its acetylated derivatives (3-ADON and 15-ADON) (Desjardins, 2006). These mycotoxins are the most common in corn, rice, and wheat worldwide (Palacios et al., 2017; Gab-Allah et al., 2021). For this reason, several countries have established regulatory limits for DON in foodstuff. For instance, in Argentina, the maximum regulatory limit of DON is established at 1000 µg/kg for wheat flour ~~*Fusarium graminearum* sensu stricto, belonging to *Fusarium graminearum* species complex is the prevalent species isolated worldwide, showing high variability in their genomic pool and toxigenic production (Ortega et al., 2016).Moreover, *F. graminearum* has the ability to produce~~ and other mycotoxins such as aurofusarin, fusarin C, nivalenol (NIV), and zearalenone (ZEA), an important mycotoxin that can cause hyperestrogenism and affects sexual reproduction in monogastric~~s~~ (Desjardins, 2006; Leslie & Summerell, 2006).

The challenge to increase agricultural production with minimum environmental impact requires maximizing resources such as solar radiation, water, and nitrogen. In this way, one of the most used systems worldwide is crop rotation as an agronomic practice to achieve ecological intensification. These agronomic management practices should follow an integrated approach to FHB management, including resistant genotypes, crop rotation, tillage practices, chemical control, sowing date, and the appropriate use of fertilizers (Lemmens et al., 2004). ~~However, the impact of N fertilization on FHB development remains unclear. Several studies performed in wheat support that the increase of the N application rate can affect FHB severity (Heier et al., 2005) and mycotoxin contamination (Krnjaja et al., 2015) depending on the environmental conditions. On the other hand, contrary results have been obtained suggesting that N fertilization does not produce changes in these parameters (Aufhammer et al., 2000). Another major factor that can affect FHB development is the sowing date. For instance, Gorczyca et al. (2018) found significant increases in FHB symptoms when the sowing date was delayed three weeks after the optimal sowing date in Austria, Slovakia, and Poland. These could be due to favorable temperature and humidity conditions, before and during the flowering stage, for fungal growth.~~

Depending on the climatic conditions that modulate the plant-pathogen interaction, FHB can affect the viability of the seeds and different aspects of the raw material in the food industry. In addition, this disease reduces yield due to a decrease in grain weight, compromising the germination and vigor of the seeds (~~Christensen & Kaufmann, 1965; Bewley et al., 2012~~Christensen & Kaufmann, 1965; Bewley et al., 2012). Furthermore, FHB reduces the starch content of the grains and degrades different gluten protein subunits, causing a lower bread-baking quality (Martínez et al., 2020). Within the biotic factors that can influence seed longevity during storage, *Fusarium* species become

relevant for wheat (~~Gilbert et al., 1997; Bewley et al., 2012~~Gilbert et al., 1997; Bewley et al., 2012), being the pathogen able to degrade gluten proteins (Birzele & Prange, 2003; Garcia-Cela et al., 2018; Bellesi et al., 2019~~ Bellesi et al., 2019~~).

The intensification of crop production to meet global food demands must address the complexity of agricultural systems, considering interactions among management factors in relation to productivity but also the integral quality of the grains. The effect of N fertilization on grain protein concentration and gliadins/glutenins composition is widely known, which is related to the bread-baking quality of wheat (Zhang et al., 2016; Tóth et al., 2019). However, the interaction between N fertilization, sowing date, and FHB development remains unclear. Therefore, the aim of this work was to analyze the effect of sowing date and nitrogen fertilization on the *F. graminearum*-wheat interaction. Furthermore, disease parameters, mycotoxin contamination, grain quality, and seed deterioration were evaluated.

2. Materials and methods

2.1 Experimental design

The experiment was carried out at the Experimental Farm of the Facultad de Agronomía, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA) (36°83´ S, 59°88´ O), Azul, Argentina. The trial was conducted during the 2018/2019 growing season under conventional tillage. The soil is a typical Argiudoll with the following features: low level of organic matter (3.84 %) (0-20 cm deep; Walkley & Black, 1934), moderately acid pH (5.97) (1:2.5, water; 0-20 cm deep), moderate availability of phosphorus (18.94 ppm) (0-20 cm deep; Bray & Kurtz, 1945), and low availability of N-nitrate (27.4 kg N/ha) (by reflectometry; 0-20 and 20-40 cm deep). Crop phenology was recorded according to Zadoks et al. (1974). A genotype of bread wheat (cv. Klein Proteo)

was chosen based on intermediate-short cycle length, good and stable fertilizer-use efficiency (Arata et al., 2017), high protein percentage (Lerner et al., 2016), and good baking quality (Quality Group 1; INASE, 2020). According to INASE (2020), this genotype was resistant to FHB, but recently Martínez et al. (2020) demonstrated that this cultivar was moderately~~Furthermore, this genotype was chosen due to the susceptibility~~ susceptible to *Fusarium* spp. under field conditions~~ (Martínez et al., 2020)~~.

For the present study, two contrasting sowing dates were chosen according to the limits of the recommended range for bread wheat (intermediate-short cycle length genotypes) for the study area (Argentinean wheat subregion IV). The sowing dates evaluated were: July 04, 2018 (SD1) and August 24, 2018 (SD2). Plant density was 250 plants m^2, and basal phosphorus (30 kg P/ha, as triple super~~-~~phosphate) and sulphur (15 kg S/ha, as CaSO4) fertilization were applied topdressing at sowing. For each sowing date, the experimental design was split-plot in a randomized complete block with three repetitions, using macro-plots of 9.5 x 1.4 m, assigning the N fertilization treatments to the main plots and *F. graminearum* treatments to the subplots. For nitrogen treatments, urea (46-0-0) was used because it is the most common N fertilizer used in the study area. Nitrogen fertilization treatments were N0 (0 kg N/ha) and N1 (180 kg N/ha, applied as topdressing with 40% applied at GS11 and 60% at GS22), while *F. graminearum* treatments were F0 (control) and F1 (inoculated). Weeds were chemically controlled by ~~the application of~~applying herbicide (6 g/ha metsulfuron-methyl + 100 cm^3/ha dicamba) at the 4-unfolded- leaves (GS14) stage of the wheat, while pest control was not required. The experiment was conducted without supplemental irrigation or fungicide treatments.

A mixture of four *F. graminearum* (3.4, 88.1, 92.2, and 129.1 isolates) previously isolated and morphologically and molecularly identified by Castañares et al. (2014) were used as inoculum based on mycotoxin *in vitro* production. The inoculum was produced by placing

individual agar plugs with mycelium in Petri dishes (90 mm) with potato dextrose agar 2% (PDA). The ~~time of incubation~~incubation time was 7 days at 25 ± 2°C under 12 h each of light and darkness. The conidial harvest was done by flooding the plates with 5 ml of sterile distilled water (SDW) and dislodging the conidia with a bent glass rod, according to Martínez et al. (2020). Wheat heads were inoculated during mid-anthesis (GS60-GS65) with conidial suspensions (1 x 10^5 conidium/ml) with Tween 20 (0.05%), applied until run-off using a hand-held garden sprayer (2 l~~L~~) with adjustable brass nozzles. The inoculation was carried out, keeping a distance between the nozzle and the spikes of around 5 cm to avoid spore dispersion. The moment of inoculation was determined according to environmental conditions such as the absence of wind (to limit the drift of the inoculum to neighboring plots) and high relative humidity (~~RH~~>80%) to avoid ~~the~~ evaporation of the inoculum. For control treatment, SDW with Tween 20 (0.05%) was used to inoculate. Daily records of mean (Tmean), minimum (Tmin) and maximum (Tmax) temperatures, precipitations ~~(Pp)~~, average relative humidity ~~(RH)~~, and global radiation ~~(GR)~~ were obtained from the Field Climate Station (iMetos 3.3, ID 0020373B, Pessl Instruments®, Austria) located near the experiments, belonging to the Regional Agrometeorology Center (FAA-UNCPBA 2020). At the time of inoculation, the intercepted photosynthetically active radiation (IPAR) by the crop was determined with a ceptometer (BAR-RAD 100, Cavadevices®, Argentina) as described by Lázaro et al. (2010), and the chlorophyll content in flag leaf was estimated (SPAD-502 Plus, Minolta®, Japan). At maturity (GS94), spike samples along the five central rows of each subplot were manually harvested and mechanically threshed.

2.2 Disease parameters and mycotoxin contamination

Disease assessment was conducted at 21 days post-inoculation (dpi). Incidence (number of spikes with symptoms) and severity (number of spikelets with symptoms per spike) were measured by counting the number of symptomatic spikelets. Forty spikes were selected at random in each subplot, and typical lesions (bleaching spikelets) were registered (Campbell & Lipps, 1998). Three indices were calculated: (i) FHB index (FHB= Incidence*Severity/100) (Snijders, 1990); (ii) ISK index [ISK= 0.3 x Incidence + 0.3 x Severity + 0.4 x *Fusarium* Damaged Kernels (FDK- as percentage of symptomatic kernels)] (Kolb ~~&and~~ Boze, 2003); (iii) DON content, incidence, severity, and kernel damage (DISK) index (DISK= 0.2 x Incidence + 0.2 x Severity + 0.3 x FDK + 0.3 x DON concentration) (Gilbert & Woods, 2006).

After harvest, the grain samples were quartered (Cereal Tools®, Argentina) and milled with a high-speed disintegrator FW-110 (Arcano©; Pasteur Instrumental, Argentina) for toxin analyses. For each sample (10 g), a volume of 20 ml of extraction solvent (CH_3CN/H_2O/HAc 79 + 20 + 1) was added. To facilitate extraction, the samples were homogenized with an Ultra-turrax® for 3 min and sonicated for 60 min, then centrifuged for 5 min at 3000 rpm. For each sample, a 10 ml volume of extract was transferred into a glass vial and evaporated dryness at 45 °C under a stream of N_2. ~~Samples were resuspended in methanol/water (70:30) and filtered through a 0.22-mm nylon filter before analysis~~Before analysis, samples were resuspended in methanol/water (70:30) and filtered through a 0.22-mm nylon filter. Trichothecenes identification and quantification were performed using ~~a~~ high-performance liquid chromatography coupled with linear trap quadrupole tandem mass spectrometry (HPLC MS^n), according to Arroyo-Manzanares et al. (2015) and Castañares et al. (2019) with some modifications. The analyses were conducted in a Thermo~~–~~Scientific™~~®~~ (Massachusetts, US) system consisting of a degasser, quaternary pump, column oven, and an Thermo Scientific™ LTQ XL~~®~~ ion trap

mass spectrometer. Chromatographic separations were performed with a C18 100 2.1 mm Hypersil® ODS (Thermo Scientific™ BDS, 5 mm particle size) column. A solution of ammonium formate (10 mM) and acetonitrile was used as a mobile phase. Samples (10 µl) were analyzed at a flow rate of 0.2 ml/min at 45°C. The quantitative determination was carried out using the following Sigma-Aldrich Co (Missouri, US) ~~®~~ standards: B-trichothecene mix (part number: 34134). The limits of quantification were 1.00 ng/g, and the limits of detection were 0.50 ng/g for 3-ADON, DON, 15-ADON, and ZEA. For NIV, the limit of quantification was 30.00 ng/g, and the ~~limit of detection~~detection limit was 10.0 ng/g.

2.3 Grain weight, quality parameters, and gluten composition

Grain weight ~~(GW)~~ was determined by counting 1000 wheat grains. The number of grains per unit area was estimated by the data collected to calculate the disease parameters. Subsequently, grain yield was estimated by multiplying the grain weight~~GW~~ by the number of grains, ~~and it was~~ expressed in g/ m^{-2}. Grain quality parameters, including protein percentage ~~(Pro%)~~, gluten percentage ~~(G%)~~, and test weight ~~(TW),~~ were analyzed using whole grain samples by Near-Infrared Transmission (NIT) Spectroscopy (AgriCheck®, Bruins Instruments, Germany). Also, on wholemeal samples, the sodium dodecyl sulfate sedimentation test (SDSS) (Dick & Quick, 1983) and the solvent retention capacity (SRC) in four solvents (distilled water, WRC; lactic acid 5% v/v, LARC; sodium carbonate 5%w/v, SCRC; and sucrose 50%w/v, SuRC) (AACC method 56-11) (Gaines, 2000) were evaluated.

Regarding gluten composition, glutenins and gliadins were extracted by the sequential extraction method, according to Gupta & MacRitchie (1991). Gliadins were extracted with propanol (50%) from wholemeal flour. Glutenins were solubilized from the remaining

pellet by further extraction with propanol (50%), Tris (1 M, pH = 8), and dithiothreitol and were alkylated with 4-vinyl pyridine (1.4%). These proteins were separated by SDS-PAGE (T=13.5%). ~~The~~ Vertical gels ~~was~~were run at constant current (40 mA) and variable voltage for approximately 4 h. Gels were ~~-~~stained with 0.05% Coomassie Brilliant Blue R250 for 24 h, distained in TCA (trichloroacetic acid) 12% for 48 h, and finally washed in distilled water for 24 h. The high molecular weight glutenin subunits (HMW) proteins were identified~~, according to Payne et al. (1987),~~ assigning numbers to the subunits according to an increase of their electrophoretic mobility (Payne et al., 1987). ~~while the low molecular weight glutenin subunits (LMW) protein fractions according to Lerner et al. (2009).~~ Common wheat genotype Chinese Spring was used as a standard for assessing the electrophoretic mobilities of proteins. The resulting gels were scanned and analyzed using TotalLab (v1.10) software to measure the intensity of the pixel as an abundance indicator. Background subtraction was applied to avoid variability caused by the staining process. Then, the ratio between contents of gliadins and glutenins (GLI/GLU), the ratio between HMW/LMW, the contents of different glutenin subunits encoded by *Glu-1* locus relative to total high molecular weight glutenins (Glu-A1x/HMW, Glu-B1x/HMW, Glu-B1y/HMW, Glu-D1x/HMW, Glu-D1y/HMW) and the ratio between ω-gliadins and α-β-γ-gliadins (ω-gli/α-β-γ-gli) were determined.

2.4 Seed deterioration

An accelerated aging assay was carried out for each treatment (SD x N x F) by placing seeds for 1-10 days at 45 °C in a controlled temperature chamber. Seeds were put in tightly closed boxes (tripled) with free water at the bottom to obtain 100% relative humidity~~RH~~. ~~Every two days, water content (WC) and germination from a sampled subset of caryopses~~

~~were assessed~~Water content (WC) and germination from a sampled subset of caryopses were assessed every two days. The water content was determined in ten caryopses by the gravimetric method and was expressed as a percentage on a dry basis. The caryopses were weighed fresh ~~and~~ after drying at 105 °C for 48 h~~ours~~ (ISTA, 2017). Germination was tested by placing 25 subsampled caryopses in Petri dishes (90 mm) with 5 ml of distilled sterilized water and incubating at 20 °C. Germination counts were performed every two days for ~~10~~ ten days, counting the seeds with normal radicle development. Seeds with abnormalities or non-germinate seeds were counted as dead (all treatments at the beginning of the aging test exceeded 95% germination).

An electrolyte leaching test was carried out by soaking 50 seeds in a 100 ml glass beaker containing 50 ml of distilled water. Beakers were covered with plastic film to reduce evaporation and ~~were then~~ placed in an incubator at 25 °C for 24 h~~ours~~. The electrical conductivity (EC) of seed leachates was measured using an electrical conductivity meter and reported as µS cm/g. Measurements were made at the beginning (EC0) and ten days of the accelerated aging assay (EC10).

2.5 Statistical analysis

The effects of sowing date (SD), N fertilization (N), *F. graminearum* (F), and their interactions were analyzed by ANOVA and Duncan test (α=0.05). The contribution of each source of variation (S.V.) was expressed as the percentage of the sum of squares with respect to the model (SS%). Block by main plot interaction as error term for the effect assigned to the main plot was used. Cluster analysis (average linkage, Euclidean distance)~~,~~ and Mantel test between Euclidean distance matrices were also performed. The InfoStat statistical package was used (Di Rienzo et al., 2017).

Regarding accelerated aging tests, in each treatment combination (SD x N x F) and sampling time during aging, the germination dynamics were plotted in days. Then, a Gomperz-type sigmoid model was adjusted, and the time to reach 50% of total germination (T50) was calculated manually, by using the GraphPad Prism® graphical-statistical software. Viability was taken as the maximum germination reached during the 10 ten days of incubation. Finally, for each treatment, the variables T50, viability, and WC water content were plotted as a function of aging days, and quadratic models were adjusted.

3. Results and discussion

3.1 Climatic conditions and crop parameters

In the present study, the effect of nitrogen fertilization in *F. graminearum*-bread wheat interaction in two sowing dates was evaluated.

As expected, the environmental conditions differed between these sowing dates. Anthesis (GS65) occurred at 123 and 85 days after sowing for SD1 and SD2, respectively. The environmental conditions were different between sowing dates, both in pre- and post-anthesis (Fig. 1). Average Tmean, accumulated precipitations Pp, and accumulated global radiation GR between sowing and anthesis were 10.8 °C, 224 mm, and 1589 MJ/m^{2}d for SD1; while they were 13.9 °C, 254 mm, and 1458 MJ/m^{2}d for SD2. In comparison, average Tmean, accumulated precipitations Pp, and average relative humidity RH between anthesis (inoculation) and symptom evaluation (21 dpi) were 18.3 °C, 157 mm, and 72% for SD1; while they were 17.2 °C, 78 mm and 68% for SD2. Also, the average Tmean during the entire post-anthesis period was 19.1 and 19.2 °C for SD1 and SD2, respectively. Short-term differences were observed in average Tmean, accumulated precipitations Pp,

and average relative humidity~~RH during the~~ 72 h after anthesis (inoculation): 17.6 °C, 0.1 mm and 70% for SD1, and 16.2 °C, 42 mm and 67% for SD2.

~~-~~The effects of temperature throughout the growing period and of the photoperiod in pre-anthesis are widely known. Slafer (2003) demonstrated an increase in plant development rates and a decrease in the duration of each phenological phase, especially on pre-anthesis, by delaying the sowing date. This leads to structural and physiological changes in the crop at the time of inoculation (anthesis). ~~Our results showed that radiation interception and chlorophyll content in the flag leaf were higher at the early sowing date than under late sowing conditions.~~

Our results showed that radiation interception (IPAR: 0.98 *vs.* 0.56) and chlorophyll content (SPAD: 41.17 *vs.* 32.22) in the flag leaf were higher at the early sowing date than under late sowing conditions. At the same time, nitrogen fertilization significantly improved these parameters at both sowing dates:~~At the time of inoculation, IPAR and SPAD values were significantly higher, on average, for SD1 than for SD2 (IPAR: 0.98 *vs.* 0.56, SPAD: 41.17 *vs.* 32.22). Furthermore, N fertilization significantly improved IPAR and SPAD~~ for SD1 (IPAR: 0.96 *vs.* 0.82, SPAD: 45.06 *vs.* 37.28) and for SD2 (IPAR: 0.59 *vs.* 0.52, SPAD: 36.39 *vs.* 28.05). ~~As expected, the environmental conditions differed between these sowing dates. At the same time, nitrogen fertilization significantly improved these parameters at both sowing dates.~~ By contrast, the estimated grain yield at harvest was higher in SD2 (320 ± 17.29 g/-m^{-2}) than in SD1 (252.39 ± 13.25 g/-m^{-2}), which could be due to the higher rainfall accumulated during the critical period for yield definition (168 *vs.* 137 mm) (Fischer, 1985). These values represented 30 and 44% of the total precipitation accumulated during the crop cycle for SD1 and SD2, respectively.

~~). However, at harvest, the estimated grain yield was 252.39 ± 13.25 g m^{-2} and 320 ± 17.29 g m^{-2}, on average, for SD1 and SD2, respectively.~~

3.2 Disease parameters and mycotoxin contamination

The three-way interaction of SD x N x F was significant for the incidence of FHB (Table A.1). A lower value in the non-inoculated treatment versus the inoculated treatment regardless of N levels or sowing date was observed. Differences between non-inoculated and inoculated were largest for the early sowing date with the higher rate of N, lowest for the early sowing date, and low nitrogen rate. FHB development can be affected by increasing N rates, modifying the crop canopy (e.g., leaf density), thus altering the canopy microclimate (Lemmens et al., 2004). ~~In our study, T~~~~there was~~Our study showed a significant difference between N rates with the early sowing data and when plots were inoculated, whereby incidence was increased with the higher N rate (Fig. 2). For severity (3.17 *vs.* 10.17%), FHB index (1.49 *vs.* 6.63%), and ISK (12.25 *vs.* 28%), only the inoculated treatment significantly increased these parameters, while DISK was 3-fold higher for SD1 (8.05) than for SD2 (33.5). In addition, N fertilization increased FDK by 0.38% on average, while F treatment decreased by 0.74%, this parameter only for SD1 (Table A.1). Several authors have found contrasting results related to the effect of N on FHB. Lori et al. (2009) observed that in a year with climatic conditions favorable to natural disease development, the N application (90 kg/ha) did not affect the incidence, severity, and FHB index. Krnjaja et al. (2015) reported that N at higher rates (75 and 150 kg/ha) had no significant impact on FHB index in winter wheat artificially inoculated with *F. graminearum* under field conditions.

Nonetheless, Heier et al. (2005) observed that N doses similar to those applied in our study increased FHB severity in a susceptible winter wheat genotype under natural infection conditions. Lemmens et al. (2004) found that increasing N rates may increase FHB intensity in field experiments with artificial inoculation. Similar results were found in our

experiments for FDK that increased due to N fertilization, suggesting that the infection capacity of the pathogen could be influenced by the crop structure and their physiological condition. Moreover, ~~I~~it is known that high relative humidity is necessary for successful FHB infections in cereal spikes, promoting the formation of macroconidia and ascospores (Sutton, 1982). Furthermore, long humid periods may promote inoculum production on crop debris and the soil surface (Fauzi & Paulitz, 1994). ~~-~~In our study, the average relative humidity, mean temperature, and accumulated precipitations between anthesis and symptom evaluation were higher in SD1 than ~~in~~ SD2. This fact could explain the higher incidence values observed in SD1. In this way, Xu & Nicholson (2009) reported that warm and humid conditions were favorable for *F. graminearum* infection. Cowger et al. (2009) demonstrated that post-anthesis moisture significantly increases infection, 4-fold the mean of disease incidence and 8-fold the disease severity after 10-20 post-anthesis moisture days.

As regard to mycotoxins, ~~S~~sowing date was the only treatment that had a significant effect on ~~mycotoxin~~ the contamination with DON, 3-ADON, ZEA, and NIV, being their mean values significantly higher for SD2 than for SD1 (Table 1). The 15-ADON was not detected in any trial.

Interestingly, the highest severity values found in SD2 were closely related to a significant increase in the mycotoxin contamination, in the order of 4 to 9-fold depending on the toxin. ZEA was the mycotoxin found in the highest concentration, followed by NIV, DON, and 3-ADON, while 15-ADON was not detected. ~~Similarly,~~ Gorczyca et al. (2018) reported higher mycotoxin contamination in winter wheat for delayed sowing dates ~~in durum wheat~~, depending mainly on genotype for three different weather conditions (Austria, Poland, and Slovakia). ~~Regardless of the sowing date, ZEA was the mycotoxin found in the highest concentration, followed by NIV, DON, and 3-ADON, while 15-~~

~~ADON was not detected.~~ Regarding fertilization, Lori et al. (2009) reported that the N application did not affect DON concentration, while ZEA level was higher than DON under natural FHB infection conditions in different tillage systems of Argentina. Conversely, other authors found that N fertilization could increase DON and/or ZEA levels depending on the environment (Lemmens et al., 2004), in addition to a predominance of DON over ZEA (Heier et al., 2005; Krnjaja et al., 2015).

3.3 Grain weight, quality parameters, and gluten composition

~~Grain quality was mainly affected by the sowing date~~The sowing date mainly affected grain quality, ~~having this~~~~The SD had a significant effect on~~significantly affecting test weight~~TW~~, protein percentage~~Pro%~~, and SCRC (Table A.2). P~~p~~rotein percentage ~~Pro%~~ (15.67 *vs.* 13.30~~-~~%) and SCRC (118.05 *vs.* 109.35~~-~~%) were significantly higher, while grain weight~~GW~~ (28.48 *vs.* 31.71 mg) and TW (79.24 *vs.* 84.17 kg/h~~l~~~~L~~) were significantly lower, on average, for SD1 than SD2. Regarding gluten percentage~~G%~~, SD1 showed a higher average value than SD2 (43.5 *vs.* 35.68%), with N fertilization increasing around 9.5% this parameter, on average. In addition, N fertilization ~~slightly~~ decreased SDSS from 97.63 to 96.13 mm, on average~~;~~, while no treatment effects were significant for LARC, WRC, and SuRC (Table A.2). In summary, early sowing showed a better protein and gluten concentration, while late sowing showed a better grain structure and carbohydrate quality due to a higher grain weight and test weight~~,~~ and lower SCRC. The limited carbohydrate accumulation could explain the lower yield for SD1 (Jenner et al., 1991). The last parameter is positively correlated to the amount of damaged starch of the flour (Guzmán et al., 2015). Nitrogen fertilization did not affect protein percentage, while gluten percentage was increased depending on the sowing date, which could improve

bread-making quality. However, the N application ~~slightly~~ reduced the SDSS, positively correlated with gluten strength (Dick & Quick, 1983), which could be related to a ~~slight~~ unbalance among protein fractions (Lerner et al., 2016).

With respect to gluten composition, no significant effects were observed for GLI/GLU ratio or Glu-B1y/HMW. N fertilization significantly decreased the HMW/LMW ratio~~,~~ in SD1 (SD1: N0=1.47~~-a~~, N1=1.20~~-b~~; SD2: N0=1.15~~-b~~, N1=1.28~~-b~~). Also, Glu-A1x/HMW was significantly higher for SD1 (0.21) than for SD2 (0.19), on average. The ~~effect of the inoculation treatment (F)~~inoculation treatment (F) effect on Glu-B1x/HMW depended on N supply and SD, while no treatment effect was significant for Glu-B1y/HMW. The higher rate of N significantly decreased GluD1x/HMW (SD1: N0=0.22~~-a~~, N1=0.20~~-b~~; SD2: N0=0.23~~-a~~, N1=0.24~~-a~~) and increased GluD1y/HMW (SD1: N0=0.16~~-b~~, N1=0.17~~-a~~; SD2: N0=0.16~~-b~~, N1=0.16~~-b~~), but only for the early sowing date (SD1). Regarding gliadin fraction, ω-gli/α-β-γ-gli was significantly higher for SD1 (1.01) than for SD2 (0.91), on average (Table A.3). Glutenin composition was affected by N application only at the early sowing date, increasing the proportion of HMW subunits ~~that are~~ composed of sulfur (S)-poor proteins. Otherwise, LMW subunits (sulfur (S)-rich proteins) decrease when sulfur is deficient relative to N (Flaete et al., 2005; Zörb et al., 2009). In addition, these results were related to an increase in the proportion of the subunit encoded by *Glu-D1y* locus to the detrimental of the encoded by *Glu-D1x*. Other authors reported a decrease of *x*-type/*y*-type ratio when fertilizing with N, depending on genotype and environment, although these changes in the ratio were not consistent (Pechanek et al., 1997). Also, the proportion of the subunit encoded by *Glu-A1x* locus decreased by delaying the sowing date. Notably, the inoculation with *F. graminearum* significantly affected the proportion of the glutenin subunit encoded by *Glu-B1x* locus, depending on N supply and sowing date, ~~which~~

highlightshighlighting the importance of studying the affinity of fungal proteases by this protein subunit. Regarding gliadin composition, the proportion of ω-gli decreased with respect to α-β-γ-gli by delaying the sowing date. Considering that ω-gliadins are S-poor protein (Zörb et al., 2009), just like HMW glutenin subunits, these could be explained by an increase in S availability in the soil by mineralization due to the increment in temperature associated with the late sowing date (Arata et al., 2017).

3.4 Seed deterioration

A drop in viability, an increase in WC water content, and T50 were observed in all treatments during the progress of seed aging (Fig. 3). The decrease in viability began from the sixth day, thenn progressing to the tenth day, where the loss of viability became almost total. The WC water content increased progressively along the duration of the assayassay duration, and the T50 increased from the fourth or sixth day depending on the treatment, reflecting the loss of vigor in the viable seeds. The longevity parameters of accelerated aging assays differed between sowing dates, showing that SD1 had lower average viability (non-cumulative values, 740.9 *vs.* 769) associated with higher WC water content (non-cumulative values, 165 *vs.* 161.2) and T50 (cumulative values, 23.6 *vs.* 21.2) compared to SD2. However, no clear differences were observed for N fertilization and F treatments on deterioration tests (Table A.4).

As regards seed deterioration, EC0 was significantly higher for SD2 than for SD1, on average;, while inoculation with *F. graminearum* significantly increased this parameter only at low N supply. In addition, EC10 was significantly higher for SD2 than for SD1, on average (Table 2). However, EC increased in a greater magnitude during the accelerated aging assays for SD1 than for SD2 (+54 % *vs.* +29%). SlightStatistically

differences were observed among longevity parameters of accelerated aging assays, showing an increase in seed vigor and viability by delaying the sowing date. Also, the response patterns of the parameters evaluated during ~~the progress of~~ seed aging were in line with the results reported by Lehner et al. (2008). Li et al. (2017) observed in *Vicia sativa* that higher temperatures during development generated seeds with less weight, viability, and higher EC values. ~~However, i~~In our work, both sowing dates had similar temperatures between flowering and harvest maturity and a lack of association between aging parameters and temperature during grain filling. Conversely, EC increased significantly by delaying the sowing date, both at the beginning and ten days of the accelerated aging assay. The increment in EC during this assay was more pronounced for late sowing seeds. The EC test was able to identify the seed vigor in genotypes exposed to different grain filling periods and storage conditions in sunflower (Szemruch et al., 2015).

As previously discussed, seed vigor and viability showed an inverse trend with EC between sowing dates. It is noteworthy that vigor (low T50 and water content) and viability were associated with a better grain structure (i.e., high grain weight and test weight) as reported by Li et al. (2017), while the less EC was related to variations in chemical composition (e.g., high protein percentage and gluten percentage%) and lower mycotoxin levels. The lack of *F. graminearum* effects in deterioration tests may have been caused by the storage temperature during aging. Rossi et al. (2001) observed that *Fusarium* development decreased above 30 °C in controlled conditions. According to this result, Gilbert et al. (1997) observed that the lower storage temperature increased *Fusarium* development and decreased the viability of grains.

Regarding cluster analysis, three groups of variables were analyzed: 1) Disease and mycotoxins, 2) Grain quality, and 3) Seed deterioration (Fig. 4). Even though some

differences were observed in the clustering among these groups, all the correlations between Euclidean distance matrices were significant. The association between group 1 (disease and mycotoxins) and group 3 (seed deterioration) was the highest (Fig. 4).

4. Discussion

In the present study, the effect of nitrogen fertilization in *F. graminearum* bread wheat interaction in two sowing dates was evaluated. As expected, the environmental conditions differed between these sowing dates. The effects of temperature throughout the growing period and of the photoperiod in pre-anthesis are widely known. Slafer (2003) demonstrated an increase in plant development rates and a decrease in the duration of each phenological phase, especially on pre-anthesis, by delaying the sowing date. This leads to structural and physiological changes in the crop at the time of inoculation (anthesis). Our results showed that radiation interception and chlorophyll content in the flag leaf were higher at the early sowing date than under late sowing conditions. At the same time, nitrogen fertilization significantly improved these parameters at both sowing dates. By contrast, the estimated grain yield at harvest was higher in SD2 than in SD1, which could be due to the higher rainfall accumulated during the critical period for yield definition (168 *vs.* 137 mm) (Fisher, 1985). These values represented 30 and 44% of the total precipitation accumulated during the crop cycle for SD1 and SD2, respectively.

FHB development can be affected by increasing N rates, modifying the crop canopy (e.g. leaf density), thus altering the canopy microclimate (Lemmens et al., 2004). Several authors have found contrasting results. Lori et al. (2009) observed that in a year with climatic conditions favorable to natural disease development, the N application (90 kg/ha) did not affect the incidence, severity, and FHB index. Krnjaja et al. (2015) reported that N at higher rates (75 and 150 kg/ha) had no significant impact on FHB index in winter

~~wheat artificially inoculated with *F. graminearum* under field conditions. Nonetheless, Heier et al. (2005) observed that N doses similar to those applied in our study increased FHB severity in a susceptible winter wheat genotype under natural infection conditions. Lemmens et al. (2004) found that increasing N rates may increase FHB intensity in field experiments with artificial inoculation. Similar results were found in our experiments for FDK that increased due to N fertilization, suggesting that the infection capacity of the pathogen could be influenced by the crop structure and their physiological condition.~~

~~It is known that high relative humidity is necessary for successful FHB infections in cereal spikes, promoting the formation of macroconidia and ascospores (Sutton, 1982). Furthermore, long humid periods may promote inoculum production on crop debris and the soil surface (Fauzi & Paulitz, 1994). In our study, the average relative humidity, mean temperature, and accumulated precipitations between anthesis and symptom evaluation were higher in SD1 than in SD2. This fact could explain the higher incidence values observed in SD1. In this way, Xu & Nicholson (2009) reported that warm and humid conditions were favorable for *F. graminearum* infection. Cowger et al. (2009) demonstrated that post-anthesis moisture significantly increases infection, 4-fold the mean of disease incidence and 8-fold the disease severity after 10-20 post-anthesis moisture days.~~

~~Interestingly, the highest severity values found in SD2 were closely related to a significant increase in the mycotoxin contamination, in the order of 4 to 9-fold depending on the toxin. Similarly, Gorczyca et al. (2018) reported higher mycotoxin contamination for delayed sowing dates in durum wheat, depending mainly on genotype. Regardless of the sowing date, ZEA was the mycotoxin found in the highest concentration, followed by NIV, DON, and 3-ADON, while 15-ADON was not detected. Regarding fertilization, Lori et al. (2009) reported that the N application did not affect DON concentration, while ZEA~~

level was higher than DON under natural FHB infection conditions in different tillage systems of Argentina. Conversely, other authors found that N fertilization could increase DON and/or ZEA levels depending on the environment (Lemmens et al., 2004), in addition to a predominance of DON over ZEA (Heier et al., 2005; Krnjaja et al., 2015).

Grain quality was mainly affected by the sowing date. Early sowing showed a better protein and gluten concentration, while late sowing showed a better grain structure and carbohydrate quality due to a higher grain weightGW and test weightTW, and lower SCRC. The limited carbohydrate accumulation could explain the lower yield for SD1 (Jenner et al., 1991). The last parameter is positively correlated to the amount of damaged starch of the flour (Guzmán et al., 2015). Nitrogen fertilization did not affect protein percentagePro%, while gluten percentageG% was increased depending on the sowing date, which could improve breadmaking quality. However, the N application slightly reduced the SDSS, positively correlated with gluten strength (Dick & Quick, 1983), which could be related to a slight unbalance among protein fractions (Lerner et al., 2016).

Glutenin composition was affected by N application only at the early sowing date, increasing the proportion of HMW subunits that are composed of sulfur (S) poor proteins. Otherwise, LMW subunits (sulfur (S) rich proteins) decrease when sulfur is deficient relative to N (Zörb et al., 2009). In addition, these results were related to an increase in the proportion of the subunit encoded by *Glu-D1y* locus to the detrimental of the encoded by *Glu-D1x*. Other authors reported a decrease of *x*-type/*y*-type ratio when fertilizing with N, depending on genotype and environment, although these changes in the ratio were not consistent (Pechanek et al., 1997). Also, the proportion of the subunit encoded by *Glu-A1x* locus decreased by delaying the sowing date. Notably, the inoculation with *F. graminearum* significantly affected the proportion of the glutenin subunit encoded by *Glu-B1x* locus, depending on N supply and sowing date, which highlights the importance of

~~studying the affinity of fungal proteases by this protein subunit. Regarding gliadin composition, the proportion of ω-gli decreased with respect to α-β γ-gli by delaying the sowing date. Considering that ω gliadins are S-poor protein (Zörb et al., 2009), just like HMW glutenin subunits, these could be explained by an increase in S availability in the soil by mineralization due to the increment in temperature associated with the late sowing date (Arata et al., 2017).~~

~~Slight differences were observed among longevity parameters of accelerated aging assays, showing an increase in seed vigor and viability by delaying the sowing date. Also, the response patterns of the parameters evaluated during the progress of seed aging were in line with the results reported by Lehner et al. (2008). Li et al. (2017) observed in *Vicia sativa* that higher temperatures during development generated seeds with less weight, viability, and higher EC values. However, in our work, both sowing dates had similar temperatures between flowering and harvest maturity and a lack of association between aging parameters and temperature during grain filling. Conversely, EC increased significantly by delaying the sowing date, both at the beginning and ten days of the accelerated aging assay. The increment in EC during this assay was more pronounced for late sowing seeds. The EC test was able to identify the seed vigor in genotypes exposed to different grain filling periods and storage conditions in sunflower (Szemruch et al., 2015).~~

~~As previously discussed, seed vigor and viability showed an inverse trend with EC between sowing dates. It is noteworthy that vigor (low T50 and WC) and viability were associated with a better grain structure (i.e. high grain weightGW and test weightTW) as reported by Li et al. (2017), while the less EC was related to variations in chemical composition (e.g. high protein percentagePro% and gluten percentageG%) and lower mycotoxin levels. The lack of *F. graminearum* effects in deterioration tests may have been~~

caused by the storage temperature during aging. Rossi et al. (2001) observed that *Fusarium* development decrease above 30 °C in controlled conditions. According to this result, Gilbert et al. (1997) observed that the lower storage temperature increased *Fusarium* development and decreased the viability of grains. Cluster analysis confirmed the predominant effect of sowing date on the three groups of variables. However, N fertilization was also an important determining factor for grain quality (group 2) at early sowing. Interestingly, clustering for the three groups of variables was correlated, even though for disease and mycotoxins (group 1) and seed deterioration (group 3) was strongest.

5. Conclusions

In the present work, the interaction between N fertilization and *F. graminearum* on disease parameters, mycotoxin contamination, grain quality, and seed deterioration was modified by sowing date. Under the study conditions, the early sowing date showed better values of some grain quality parameters, while the late sowing would be related to high FHB severity values and mycotoxin contamination. Nitrogen fertilization slightly reduced the sedimentation test value, although increased wet gluten percentage. Based on these results, agronomic practices such as sowing date and nitrogen fertilization and their interaction with FHB could be adjusted to produce innocuous and/or good quality grains. in each environment.

Funding

This work is partially funded by CADES, Municipalidad de Azul, Buenos Aires, Argentina.

Declarations of interest: none

Acknowledgments

We thank the Laboratory of Industrial Quality Analysis of Wheat - UNCPBA (~~http://labcit.faa.unicen.edu.ar/~~http://labcit.faa.unicen.edu.ar/), Fernández M.D., Pacheco G. for technical assistance in field experiments, and Oyarzábal M. for English assistance.

References

Arata, A.F., Lerner, S.E., Tranquilli, G.E., Arrigoni, A.C., Rondanini, D.P. (2017). Nitrogen x sulphur interaction on fertiliser-use efficiency in bread wheat genotypes from the Argentine Pampas. *Crop and Pasture Science*, *68*, 202-212. ~~https://doi.org/10.1071/CP16330.~~

Arroyo-Manzanares, N., Huertas-Perez, J.F., Gamiz-Gracia, L., García-Campaña, A.M. (2015). Simple and efficient methodology to determine mycotoxins in cereal syrups. *Food Chemistry*, *177*, 274-279. ~~https://doi.org/10.1016/j.foodchem.2015.01.040.~~

~~Aufhammer, W., Kübler, E., Kaul, H.P., Hermann, W., Höhn, D., Yi, C. (2000). Infection with head blight (*F. graminearum*, *F. culmorum*) and deoxynivalenol concentration in winter wheat as influenced by N fertilization. *Pflanzenbauwissenschaften*, *4*, 72-78.~~

Bellesi, F.J., Arata, A.F., Martínez, M., Arrigoni, A.C., Stenglein, S.A., Dinolfo, M.I. (2019). Degradation of gluten proteins by Fusarium species and their impact on the grain quality of bread wheat. *Journal of Stored Products Research*, *83*, 1-8. ~~https://doi.org/10.1016/j.jspr.2019.05.007.~~

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Bewley, J.D., Bradford, K., Hilhorst, H., Nonogaki, H. (2012). *Seeds: Physiology of Development, Germination, and Dormancy*. 3rd Edition, ed. Springer Science and Business Media.

Birzele, B., & Prange, A. (2003). *Fusarium* spp. and storage fungi in suboptimally stored wheat: mycotoxins and influence on wheat gluten proteins. *Mycotoxin Research*, *19*, 162-170. https://doi.org/10.1007/BF02942958.

Bray, R.H., & Kurtz, L.T. (1945). Determination of total, organic and available forms of phosphorus in soil. *Soil Science*, *59*, 39-45.

Campbell, K.A., & Lipps, P.E. (1998). Allocation of resources: sources of variation in *Fusarium* head blight screening nurseries. *Phytopathology 88*, 1078-1086. https://doi.org/10.1094/PHYTO.1998.88.10.1078.

Castañares, E., Albuquerque, D.R., Dinolfo, M.I., Fernández Pinto, V., Patriarca, A., Stenglein, S.A. (2014). Trichothecene genotypes and production profiles of *Fusarium graminearum* isolates obtained from barley cultivated in Argentina. *International Journal of Food Microbiology*, *179*, 57-63. https://doi.org/10.1016/j.ijfoodmicro.2014.03.024.

Castañares, E., Martínez, M., Cristos, D., Rojas, D., Lara, B., Stenglein, S.A., Dinolfo, M.I. (2019). *Fusarium* species and mycotoxin contamination in maize in Buenos Aires province, Argentina. *European Journal of Plant Pathology*, *155*, 1265-1275. https://doi.org/10.1007/s10658-019-01853-5.

Christensen, C.M., & Kaufmann, H.H. (1965). Deterioration of stored grains by fungi. *Annual Review of Phytopathology*, *3*, 69-84. https://doi.org/10.1146/annurev.py.03.090165.000441.

Cowger, C., Patton-Özkurt, J., Brown-Guedira, G., Perugini, L. (2009). Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North

Carolina winter wheat. *Phytopathology*, *99*, 320-327. ~~https://doi.org/10.1094/PHYTO-99-4-0320.~~

Desjardins, A. E. (2006). *Fusarium Mycotoxins: Chemistry, Genetics, and Biology*. St Paul, MN, US: APS.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C. W. (2017). InfoStat versión 2017. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Retrieved May 21, 2021 from: ~~http://www.infostat.com.ar~~http://www.infostat.com.ar

Dick, J.W., & Quick, J.S. (1983). A modified screening test for rapid estimation of gluten strength in early-generation durum wheat breeding lines. *Cereal Chemistry*, *60*, 315-318.

Dubin, H.J., Gilchrist, L., Reeves, J., MacNab, A. (1997). *Fusarium head scab: Global status and future prospects.* El Batan, México: CIMMYT.

FAA-UNPCBA (2020). Regional Agrometeorology Center. Retrieved May 21, 2021 from: ~~http://www1.faa.unicen.edu.ar/centro/centroreg.php~~http://www1.faa.unicen.edu.ar/centro/centroreg.php

Fauzi, M.T., & Paulitz, T.C. (1994). The effect of plant growth regulators and nitrogen on Fusarium head blight of the spring wheat cultivar Max. *Plant Disease*, *78*, 289-292.

Flaete, N. E. S., Hollung, K., Ruud, L., Sogn, T., Færgestad, E. M., Skarpeid, H. J., ... & Uhlen, A. K. (2005). Combined nitrogen and sulphur fertilisation and its effect on wheat quality and protein composition measured by SE-FPLC and proteomics. *Journal of Cereal Science*, *41*, 357-369

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Default Paragraph Font, Font: (Default) Calibri, 11 pt, English (United Kingdom)

Formatted: Font: (Default) Times New Roman, 12 pt

Fischer, R.A. (1985). Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science-Cambridge, 105*, 447-461.

Gab-Allah, M. A., Mekete, K. G., Choi, K., Kim, B. (2021). Occurrence of major type B-trichothecenes and deoxynivalenol-3-glucoside in cereal-based products from Korea. *Journal of Food Composition and Analysis, 99*, 103851.

Gaines, C.S. (2000). Report of the AACC committee on soft wheat flour. Method 56-11. Solvent Retention Capacity Profile. *Cereal Foods World, 45*, 303-306.

García-Cela, E., Kiaitsi, E., Sulyok, M., Medina, A., Magan, N. (2018). *Fusarium graminearum* in stored wheat: use of CO_2 production to quantify dry matter losses and relate this to relative risks of zearalenone contamination under interacting environmental conditions. *Toxins, 10*, 86. https://doi.org/10.3390/toxins10020086.

Gilbert, J., Tekauz, A., Woods, S.M. (1997). Effect of storage on viability of Fusarium head blight-affected spring wheat seed. *Plant Disease, 81*, 159-162. https://doi.org/10.1094/PDIS.1997.81.2.159.

Gilbert, J., & Woods, S.M. (2006). Strategies and considerations for multi-location FHB screening nurseries. In: T. Ban, J.M. Lewis, E.E. Phipps (Eds.), *The Global Fusarium Initiative for International Collaboration: A strategic planning workshop* (pp.93-102). El Batan, México: CIMMYT.

Gorczyca, A., Oleksy, A., Gala-Czekaj, D., Urbaniak, M., Laskowska, M., Waskiewicz, A., Stepien, L. (2018). Fusarium head blight incidence and mycotoxin accumulation in three durum wheat cultivars in relation to sowing date and density. *The Science of Nature, 105*, 2. https://doi.org/10.1007/s00114-017-1528-7.

Gupta, R. B., & MacRitchie, F. (1991). A rapid one-step one dimensional SDS-procedure for analysis of subunit composition of glutenin in wheat. *Journal of Cereal Science*, *14*, 105-109. https://doi.org/10.1016/S0733-5210(09)80130-6.

Guzmán, C., Posadas-Romano, G., Hernández-Espinosa, N., Morales-Dorantes, A., Peña, R.J. (2015). A new standard water absorption criteria based on solvent retention capacity (SRC) to determine dough mixing properties, viscoelasticity, and bread-making quality. *Journal of Cereal Science*, *66*, 59-65. https://doi.org/10.1016/j.jcs.2015.10.009.

Heier, T., Jain, S.K., Kogel, K.H., Pons-Kuhnemann, J. (2005). Influence of N-fertilization and Fungicide Strategies on Fusarium Head Blight Severity and Mycotoxin Content in Winter Wheat. *Journal of Phytopathology*, *153*, 551-557. https://doi.org/10.1111/j.1439-0434.2005.01021.x.

Horvat, D., Spanic, V., Dvojkovic, K., Simic, G., Magdic, D., Nevistic, A. (2015). The influence of *Fusarium* infection on wheat (*Triticum aestivum* L.) proteins distribution and baking quality. *Cereal Research Communications, 43*, 61-71. https://doi.org/10.1556/CRC.2014.0023.

INASE (2020). Instituto Nacional de Semillas. Retrieved May 11, 2021 from: https://www.argentina.gob.ar/red-de-ensayos-comparativos-de-variedades-de-trigo/grupo-de-calidad-de-trigo-panhttps://www.argentina.gob.ar/red-de-ensayos-comparativos-de-variedades-de-trigo/grupo-de-calidad-de-trigo-pan

ISTA (2017). *International rules for seed testing: The international Seed Testing Association (ISTA).* Zürichstr. 50, CH-8303 Bassersdorf, Switzerland.

Jenner C.F., Ugalde T.D., Aspinall D. (1991). The physiology of starch and protein deposition in the endosperm of wheat. *Australian Journal of Plant Physiology*, *18*, 211-226. https://doi.org/10.1071/PP9910211.

Formatted: Default Paragraph Font, Font: (Default) Calibri, 11 pt

Kolb, F.L., & Boze, L.K. (2003). An alternative to the FHB index: Incidence, severity, kernel rating (ISK) index. In: S. Canty, A. Clark, J. Mundell, E. Walton, D. Ellis, D. Van Sanford (Eds.), *Proc. 2003. Natl. Fusarium Head Blight Forum* (pp.259). East Lansing, MI: University of Kentucky.

Krnjaja, V., Mandić, V., Lević, J., Stanković, S., Petrović, T., Vasić, T., Obradović, A. (2015). Influence of N-fertilization on Fusarium head blight and mycotoxin levels in winter wheat. *Crop Protection, 67*, 251-256. https://doi.org/10.1016/j.cropro.2014.11.001.

Lázaro, L., Abbate, P.E., Cogliatti, D., Andrade, F.H. (2010). Relationship between yield, growth and spike weight in wheat under phosphorus deficiency and shading. *Journal of Agricultural Science-Cambridge, 148*, 83-93. DOI:10.1017/S0021859609990402.

Lehner, A., Mamadou, N., Poels, P., Côme, D., Bailly, C., Corbineau, F. (2008). Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageeing in wheat grains. *Journal of Cereal Science, 47*, 555-565. https://doi.org/10.1016/j.jcs.2007.06.017.

Lemmens, M., Haim, K., Lew, H., Ruckenbauer, P. (2004). The Effect of Nitrogen Fertilization on *Fusarium* Head Blight Development and Deoxynivalenol Contamination in Wheat: Impact of Nitrogen Fertilization. *Journal of Phytopathology, 152*, 1-8. https://doi.org/10.1046/j.1439-0434.2003.00791.x.

Lerner, S.E., Arata, A.F., Arrigoni, A.C. (2016). Relationship between nitrogen use efficiency and industrial quality in Argentine bread wheat varieties (*Triticum aestivum* L.) with different compositions of gluten. *Revista de Investigaciones Agropecuarias, 42*, 29-40.

~~Lerner, S. E., Kolman, M., Rogers, W.J. (2009). Quality and endosperm storage protein variation in Argentinean grown bread wheat. I. Allelic diversity and discrimination between cultivars. *Journal of Cereal Science*, *49*, 337-345. https://doi.org/10.1016/j.jcs.2008.04.003.~~

Leslie, J.F., & Summerell, B.A. (2006). *The Fusarium Laboratory Manual*. Oxford, UK: Blackwell Publishing.

Li, R., Chen, L., Wu, Y., Zhang, R., Baskin, C.C., Baskin, J.M., Hu, X. (2017). Effects of cultivar and maternal environment on seed quality in *Vicia sativa*. *Frontiers in Plant Science*, *8*, 1411. ~~https://doi.org/10.3389/fpls.2017.01411,~~

Lori, G.A., Sisterna, M.N., Sarandón, S.J., Rizzo, I., Chidichimo, H. (2009). Fusarium head blight in wheat: Impact of tillage and other agronomic practices under natural infection. *Crop Protection*, *28*, 495-502. ~~https://doi.org/10.1016/j.cropro.2009.01.012.~~

Martínez, M., Ramírez Albuquerque, L., Arata, A.F., Biganzoli, F., Fernández Pinto, V., Stenglein, S.A. (2020). Effects of *Fusarium graminearum* and *Fusarium poae* on disease parameters, grain quality and mycotoxins contamination in bread wheat (Part I). *Journal of the Science and Food Agriculture*, *100*, 863-873. ~~https://doi.org/10.1002/jsfa.10099.~~

Ortega, L.M., Dinolfo, M.I., Astoreca, A.L., Alberione, E.J., Stenglein, S.A., Alconada, T.M. (2016). Molecular and mycotoxin characterization of Fusarium graminearum isolates obtained from wheat at a single field in Argentina. *Mycological Progress*, *15*, 1. ~~https://doi.org/10.1007/s11557-015-1147-7.~~

Palacios, S.A., Erazo, J.G., Ciasca, B., Lattanzio, V.M, Reynoso, M.M., Farnochi, M.C., Torres, A.M. (2017). Ocurrence of deoxynivalenol and deoxynivalenol-3-glucoside in durum wheat from Argentina. *Foof Chemistry*, *230*, 728-734.

Payne, P.I., Nightingale, M.A., Krattiger, A.F., Holt, L.M. (1987). The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *Journal of the Science and Food Agriculture, 40*, 51-65. https://doi.org/10.1002/jsfa.2740400108.

Pechanek, U., Karger, A., Gröger, S., Charvat, B., Schöggl, G., Lelley, T. (1997). Effect of nitrogen fertilization on quantity of flour protein components, dough properties, and breadmaking quality of wheat. *Cereal Chemistry, 74*, 800-805. https://doi.org/10.1094/CCHEM.1997.74.6.800.

Placinta, C.M., D'Mello, J.P.F., MacDonald, A.M.C. (1999). A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Animal Feed and Science Technology*, 78, 21-37. https://doi.org/10.1016/S0377-8401(98)00278-8.

Reis, E.M., & Carmona, M. (2002). *Fusariosis del Trigo: Biología, Epidemiología y Estrategias Para su Manejo*. Buenos Aires, Argentina: BASF S.A.

Rossi, V., Ravanetti, A., Pattori, E., Giosuè, S. (2001). Influence of temperature and humidity on the infection of wheat spikes by some fungi causing Fusarium head blight. *Journal of Plant Pathology, 83*, 189-198.

Slafer, G.A., Miralles, D. J., Savin, R., Whitechurch, E.M., González, F.G. (2003). Ciclo ontogénico, dinámica del desarrollo y generación del rendimiento y la calidad en trigo. In: E.H. Satorre, R.L. Benech-Arnold, G.A. Slafer, E. de la Fuente, D.J. Miralles, M.E. Otegui, R. Savin (Eds.), *Producción de Granos. Bases Funcionales para su Manejo* (pp.99-132). Buenos Aires, Argentina: Editorial Facultad de Agronomía.

Snijders, C.H.A. (1990). Genetic variation for resistance to Fusarium head blight in bread wheat. *Euphytica, 50*, 171-179. https://doi.org/10.1007/BF00023642.

Sutton, C. (1982). Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Canadian Journal of Plant Pathology, 4*, 195-209. https://doi.org/10.1080/07060668209501326.

Szemruch, C., Del Longo, O., Ferrari, L., Renteria, S., Murcia, M., Cantamutto, M., Rondanini, D. (2015). Ranges of Vigor Based on the Electrical Conductivity Test in Dehulled Sunflower Seeds. *Research Journal of Seed Science, 8*, 12-21. http://dx.doi.org/10.3923/rjss.2015.12.21.

Tóth, B., van Biljon, A., Moloi, M. J., & Labuschagne, M. (2019). Effects of different fertilization levels on the concentration of high molecular weight glutenin subunits of two spring, hard red bread wheat cultivars. *Cereal Chemistry, 96*, 1004-1010.

Walkley, A., & Black, I., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science, 37*, 29-38.

Xu, X., & Nicholson, P. (2009). Community Ecology of Fungal Pathogens Causing Wheat Head Blight. *Annual Review of Phytopathology, 47*, 83-103. https://doi.org/10.1146/annurev-phyto-080508-081737.

Zadoks, J.C., Chang, T.T., Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Research, 14*, 415-421.

Zhang, Y., Dai, X., Jia, D., Li, H., Wang, Y., Li, C., Xu, H., He, M. (2016). Effects of plant density on grain yield, protein size distribution, and breadmaking quality of winter wheat grown under two nitrogen fertilisation rates. *European Journal of Agronomy, 73*, 1-10. https://doi.org/10.1016/j.eja.2015.11.015.

Zörb, C., Steinfurth, D., Seling, S., LangenkÄmper, G., Koehler, P., Wieser, H., Lindauer, M.G., Mühling, K.H. (2009). Quantitative Protein Composition and Baking Quality of Winter Wheat as Affected by Late Sulfur Fertilization. *Journal of*

Agricultural and Food Chemistry, 57, 3877-3885. https://doi.org/10.1021/jf8038988.

Figure captions

Fig. 1. Maximum (Tmax), medium (Tmean) and minimum (Tmin) temperatures, precipitation ~~(Pp)~~, global radiation (GR, only before inoculation), and average relative humidity (relative humidity~~RH~~, only after inoculation) during the crop cycles. Sowing and inoculation dates were indicated (SD1, early sowing date; SD2, late sowing date).

Fig. 2. Incidence values (%) for SD1 and SD2 experiments for N0F0, N0F1, N1F0, and N1F1 (**N0**=0 kg N/ha; **N1**= 180 kg N/ha; **F0**= control; **F1**= *F. graminearum*)

Fig. 3. Viability, water content ~~(WC)~~, and T50 (d) in function of days after accelerated aging for each treatment (SD, sowing date; N, nitrogen; F, *Fusarium*). Each point of viability and water content ~~WC~~ represent the mean (n=3) and, in T50, the estimated value of the sigmoid germination model.

Fig. 4. Cluster analysis (average linkage, Euclidean distance) for the combination of treatments (SD, sowing date; N, nitrogen; F, *Fusarium*) on three groups of variables: 1) Disease and mycotoxins (incidence, severity, FHB index, FDK, ISK, DISK, DON, 3-ADON, ZEA, NIV), 2) Grain quality (grain weight~~GW~~, TW, protein percentage~~Pro%~~, gluten percentage~~G%~~, SDSS, SCRC, LARC, WRC, SuRC, GLI/GLU, HMW/LMW, ω-gli/α-β-γ-gli, GLU-A1x/HMW, GLU-B1x/HMW, GLU-B1y/HMW, GLU-D1x/HMW, GLU-D1y/HMW), and 3) Seed deterioration (Viability, water content~~WC~~, T50, EC0, EC10). Mantel tests between Euclidean distance matrices are shown.

Figure 1

Click here to access/download;Figure;Figure 1.jpg

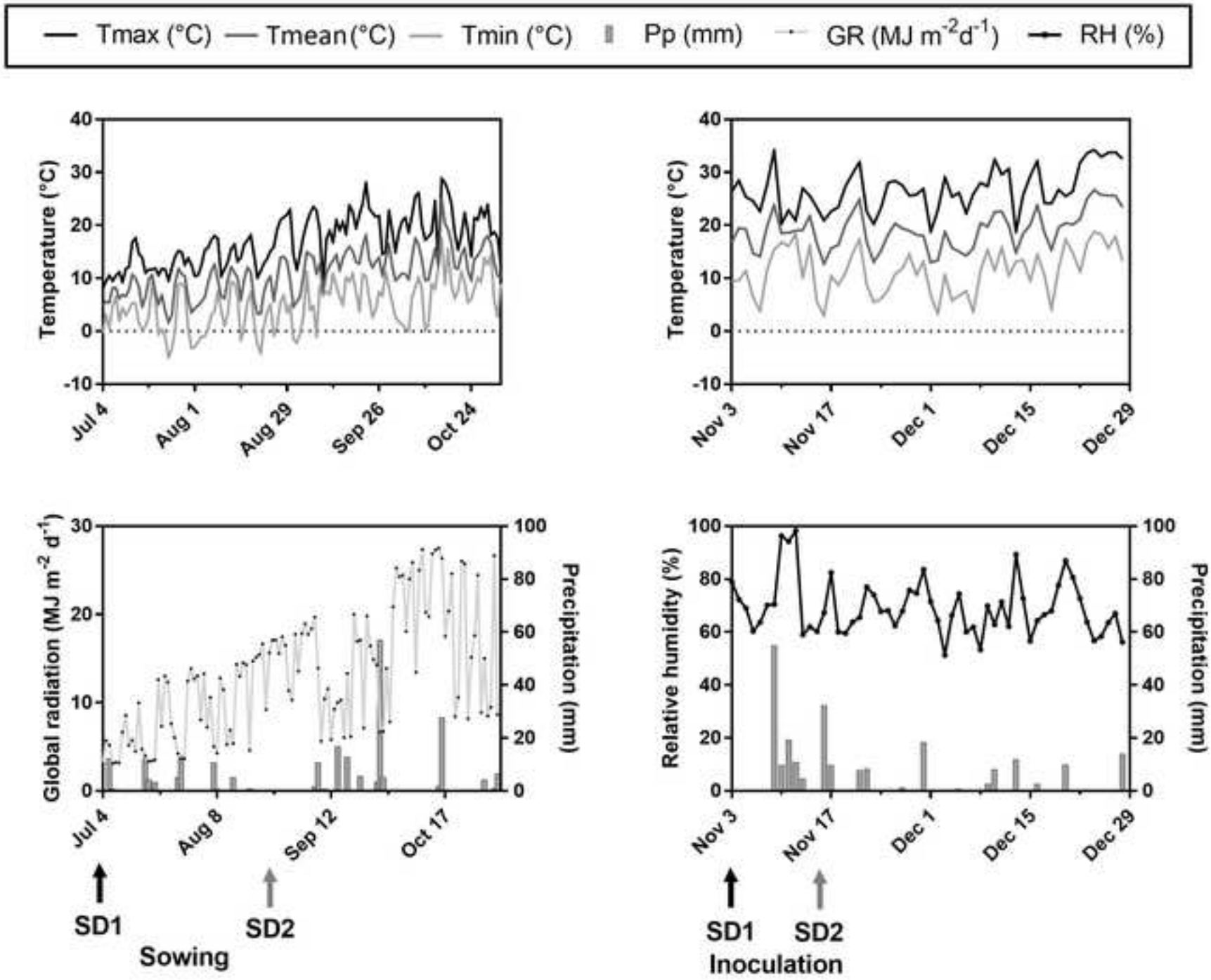


Figure 2

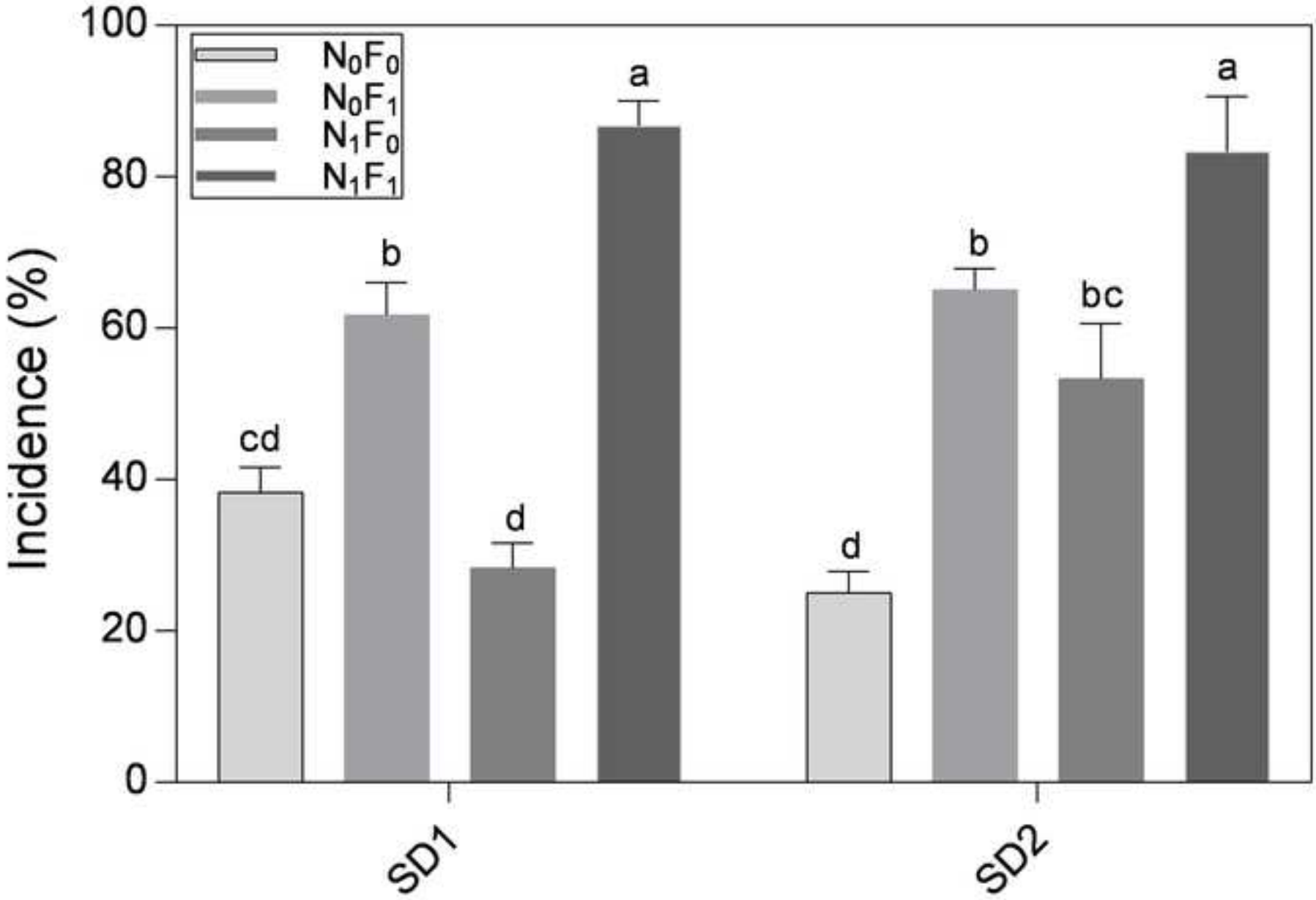


Figure 3

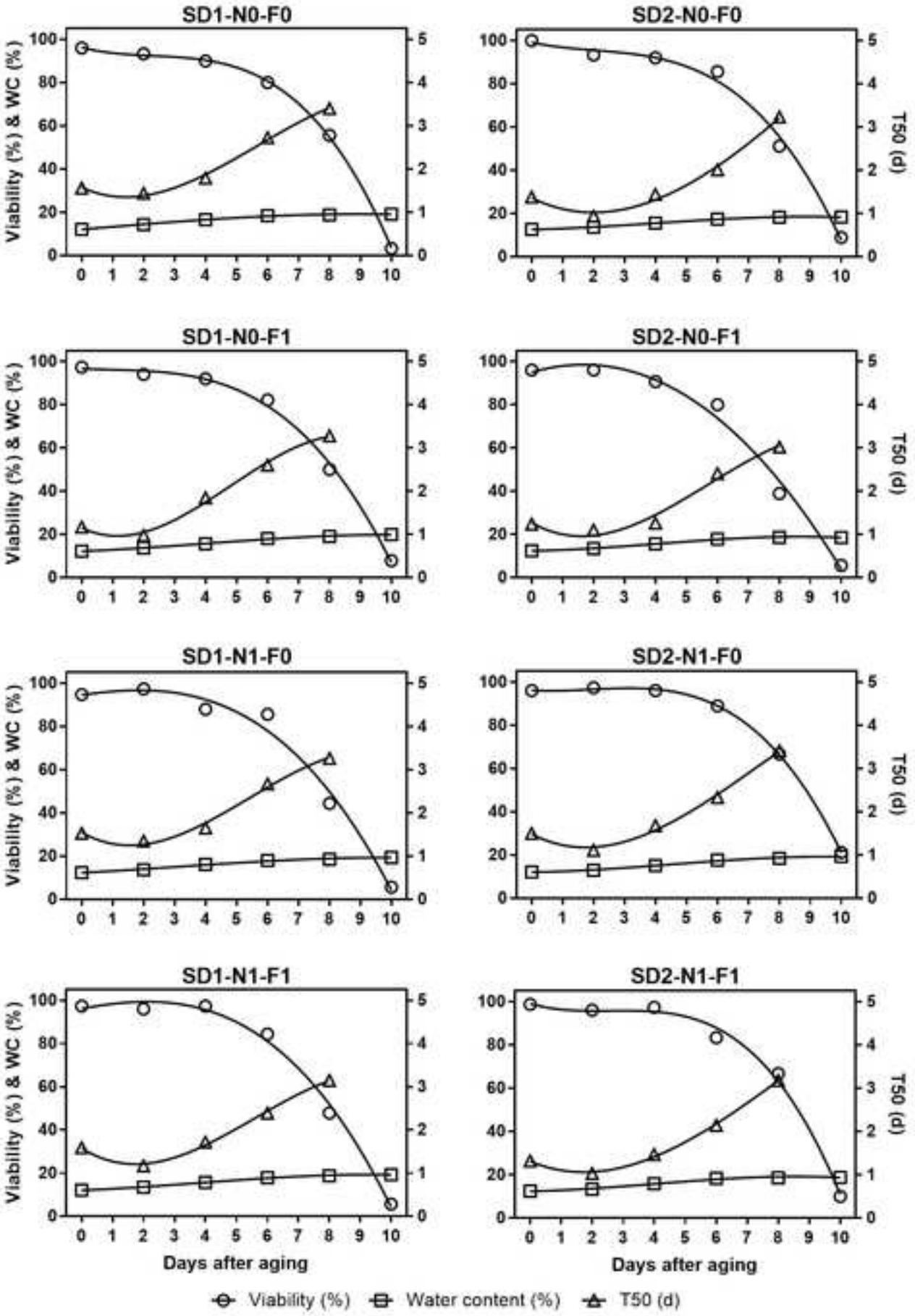
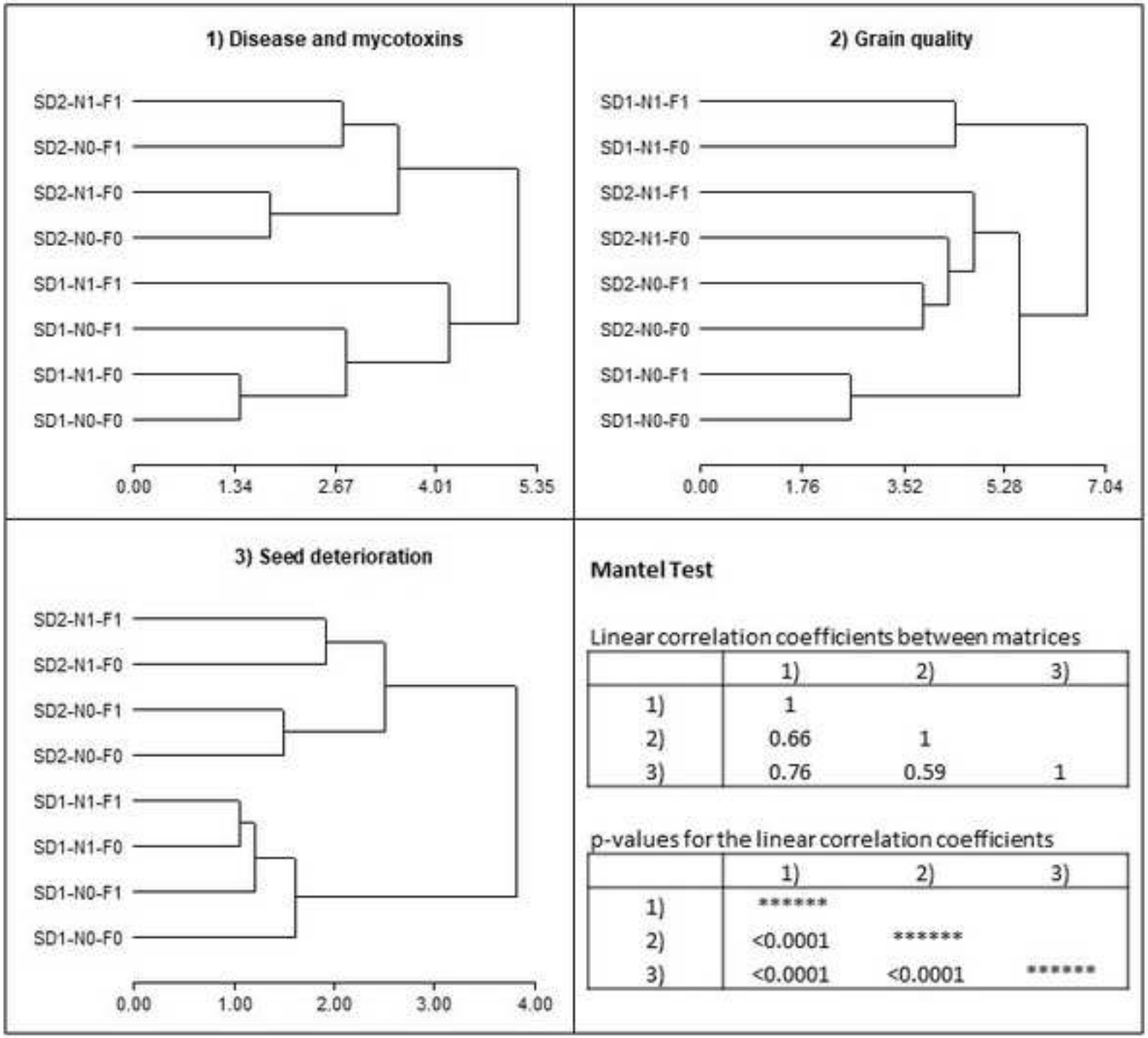


Figure 4



Mantel Test

Linear correlation coefficients between matrices

	1)	2)	3)
1)	1		
2)	0.66	1	
3)	0.76	0.59	1

p-values for the linear correlation coefficients

	1)	2)	3)
1)	******		
2)	<0.0001	******	
3)	<0.0001	<0.0001	******

Table 1. Effect of sowing date, nitrogen fertilization and *Fusarium graminearum* on mycotoxins contamination.

~~Percentage of the sum of squares respect to the model (SS%) and significance level of each source of variation (SV: SD, sowing date; N, nitrogen; F, *Fusarium*) for mycotoxin concentration (DON, 3-ADON, ZEA and NIV). Mean values were indicated for the significance SV with the highest level of a significant interaction.~~

Source of variation~~SV~~	DON (ng g^{-1})			3-ADON (ng g^{-1})			ZEA (ng g^{-1})			NIV (ng g^{-1})		
	SS%	p-value		SS%	p-value		SS%	p-value		SS%	p-value	
SD	**88.8**	**<0.0001**		**85.9**	**<0.0001**		**52.5**	**0.0222**		**86.6**	**<0.0001**	
N	0.3	0.1844		0.9	0.2871		0.6	0.5502		0.0	0.9191	
F	0.3	0.7338		0.2	0.7976		4.7	0.4459		1.5	0.4101	
SD*N	0.0	0.9946		0.3	0.7177		13.1	0.2151		1.1	0.4662	
SD*F	1.4	0.4601		1.0	0.5232		20.7	0.1254		1.1	0.4680	
N*F	1.0	0.5224		1.2	0.4801		4.5	0.4551		0.1	0.8343	
SD*N*F	0.1	0.8249		0.0	0.9236		0.1	0.9284		0.0	0.9922	
Means	SD1	39.35	b	SD1	10.34	b	SD1	249.46	b	SD1	55.89	b
	SD2	166.51	a	SD2	47.94	a	SD2	1516.92	a	SD2	504.78	a

SV: SD, sowing date; N, nitrogen; F, *Fusarium*. ~~) for mycotoxin concentration (DON, 3-ADON, ZEA and NIV). Mean values were indicated for the significance SV with the highest level of a significant interaction.~~*Means with the same letter are not significantly different between treatments ($p<0.05$, Duncan test).

Formatted: Font: Not Bold
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Centered
Formatted: Font: 12 pt

Table 2. Effect of sowing date, nitrogen fertilization and *Fusarium graminearum* on electrical conductivity.

Percentage of the sum of squares respect to the model (SS%) and significance level of each source of variation (SV: SD, sowing date; N, nitrogen; F, *Fusarium*) for electrical conductivity at the beginning (EC0) and at ten days of the accelerated aging assay (EC10). Mean values were indicated for the significance SV with the highest level of a significant interaction.

Source of variationSV	EC0 (µS cm.g^{-1})			EC10 (µS cm.g^{-1})		
	SS%	p-value		SS%	p-value	
SD	**90.6**	**<0.0001**		**78.9**	**0.0004**	
N	0.9	0.5016		0.3	0.7871	
F	0.5	0.2847		2.1	0.4502	
SD*N	1.9	0.0517		6.5	0.1937	
SD*F	0.0	0.95		0.5	0.7091	
N*F	**2.5**	**0.0281**		1.0	0.5946	
SD*N*F	0.5	0.2847		0.6	0.6784	
Means	SD1	60.33	b			
	SD2	91.33	a	SD1	93.14	b
	N0F0	73.58 b	b			
	N0F1	81.12 a	a	SD2	117.46	a
	N1F0	75.75 ab	ab			
	N1F1	72.88 b	b			

Percentage of the sum of squares respect to the model (SS%) and significance level of each source of variation (SV: SD, sowing date; N, nitrogen; F, *Fusarium*;) EC0: for electrical conductivity at the beginning; (EC10: electrical conductivity) and atat ten days of the accelerated aging assay (EC10). Mean values were indicated for the significance SV with the highest level of a significant interaction.*Means with the same letter are not significantly different between treatments (p<0.05, Duncan test).

Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Centered
Formatted: Font: 10 pt
Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Font: 10 pt
Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Font: 10 pt
Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Font: 10 pt
Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Font: 10 pt
Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Font: 10 pt
Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Font: 10 pt
Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Font: 10 pt

Declaration of Competing Interest

The authors have no conflict of interest.

Author statement

Gonzalo J. Arata*: Conceptualization, Methodology, Data curation, Writing - original draft; Mauro Martínez*: Conceptualization, Methodology, Data curation, Writing - original draft; Constanza Elguezábal: Data curation, Writing - original draft; Dante Rojas: Formal analysis, Writing - review & editing; Diego Cristos: Formal analysis, Writing - review & editing; María I. Dinolfo: Investigation, Funding acquisition, Supervision, Writing - review & editing; Agustín F. Arata: Investigation, Funding acquisition, Supervision, Writing - review & editing.

Table A.1. Effect of sowing date, nitrogen fertilization, and *Fusarium graminearum* on disease parameters. ~~Percentage of the sum of squares respect to the model (SS%) and significance level of each source of variation (SV: SD, sowing date; N, nitrogen; F, *Fusarium*) for disease parameters: incidence, severity, FHB index, FDK, ISK, and DISK). Mean values were indicated for the significance SV with the highest level of a significant interaction.~~

Source of variation~~SV~~	Incidence (%)			Severity (%)			FHB Index			FDK (%)			ISK (%)			DISK		
	SS%	p-value		SS%	p-value		SS%	p-value		SS%	p-value		SS%	p-value		SS%	p-value	
SD	0.9	0.4213		2.9	0.1852		0.0	0.9958		**60**	**0.0001**		0.4	0.4825		**88.8**	**<0.0001**	
N	12.0	0.0513		15.3	0.1844		16.4	0.1891		**10**	**0.0404**		13.8	0.0745		0.3	0.1791	
F	73.5	<0.0001		**60.6**	**<0.0001**		**48.3**	**0.006**		10	0.0795		**71.7**	**<0.0001**		0.2	0.7575	
SD*N	3.4	0.0432		1.2	0.3672		0.0	0.9368		3.7	0.1763		2.5	0.1166		0.0	0.9896	
SD*F	0.9	0.4213		0.0	0.8785		2.7	0.4429		**10**	**0.0403**		0.4	0.4825		1.4	0.4585	
N*F	1.7	0.0996		5.8	0.0651		13.4	0.1049		0.8	0.5152		4.1	0.0504		1	0.5181	
SD*N*F	**6.8**	**0.0075**		0.6	0.5439		0.6	0.7397		0.0	0.9253		3.7	0.0599		0.1	0.8181	
Means	SD1N0F0	38.33	cd							N0	1.16	b						
	SD1N0F1	61.67	b	F0	3.17	b	F0	1.49	b	N1	1.54	a	F0	12.25	b	SD1	8.05	b
	SD1N1F0	28.33	d															
	SD1N1F1	86.67	a							SD1F0	2.22	a						
	SD2N0F0	25	d							SD1F1	1.48	b						
	SD2N0F1	65	b	F1	10.17	a	F1	6.63	a	SD2F0	0.82	c	F1	28	a	SD2	33.5	a
	SD2N1F0	53.33	bc							SD2F1	0.88	c						
	SD2N1F1	83.33	a															

Formatted: Font: Bold

SD, sowing date; N, nitrogen; F, *Fusarium*; ~~) for disease parameters: incidence, severity, FHB index, FDK, ISK, and DISK). Mean values were indicated for the significance SV with the highest level of a significant interaction.~~*Means with the same letter are not significantly different between treatments ($p<0.05$, Duncan test).

Table A.2. Effect of sowing date, nitrogen fertilization, and *Fusarium graminearum* on grain quality parameters.

~~Percentage of the sum of squares respect to the model (SS%) and significance level of each source of variation (SV: SD, sowing date; N, nitrogen; F, *Fusarium*) for: grain weight (GW), test weight (TW), protein percentage (%), gluten percentage (G%), sedimentation test (SDSS), and solvent retention capacities (sodium carbonate, SCRC; lactic acid, LARC; distilled water, WRC; sucrose, SuRC). Mean values were indicated for the significance SV with the highest level of a significant interaction.~~

Source of variation~~SV~~	Grain weight~~W~~ (g)		Test weight~~W~~ (kg hl^{-1})		Protein%		Gluten%		SDSS (mm)		SCRC		LARC		WRC		SuRC	
	SS%	p-value	SS%	p-value	SS%	p-value	SS%	p-value	SS%	p-value	SS%	p-value	SS%	p-value	SS%	p-value	SS%	p-value
SD	**69.3**	**<0.0001**	**90.5**	**<0.0001**	**76.2**	**<0.0001**	**80.1**	**<0.0001**	10.7	0.1851	31.3	0.0134	7.3	0.4448	5.8	0.5194	10.5	0.3604
N	19.1	0.0904	3.1	0.1916	18.7	0.0565	**16.8**	**0.0335**	**34.8**	**0.0023**	2.6	0.7061	15.8	0.3162	8.4	0.2575	3.1	0.7362
F	0.3	0.4925	0.3	0.5048	0.1	0.7048	0.0	0.924	0.4	0.7833	3.0	0.3850	11.4	0.3422	20.8	0.232	5.4	0.5076
SD*N	**3.3**	**0.0457**	0.1	0.6685	1.8	0.1406	1.1	0.1688	13.0	0.1479	2.7	0.4131	19.3	0.2234	9.7	0.4069	0.6	0.8181
SD*F	0.9	0.2609	0.8	0.2771	0.0	0.7902	0.0	0.7569	0.1	0.8905	5.5	0.2482	0.5	0.8353	0.2	0.8978	2.0	0.6838
N*F	0.0	0.8174	0.1	0.6396	0.0	0.9349	0.0	0.9198	1.0	0.6806	4.2	0.3122	2.0	0.6842	9.0	0.4254	0.0	0.9768
SD*N*F	0.3	0.4925	0.1	0.6828	0.0	0.9519	0.0	0.8096	10.7	0.1851	16.3	0.0585	21.9	0.1968	8.2	0.4443	6.5	0.4708
Means	SD1N0	29.68 c	SD1	79.24 b	SD1	15.67 a	SD1	43.50 a	N0	97.63 a	SD1	118.05 a	---		---		---	
	SD1N1	27.28 d					SD2	35.68 b										
	SD2N0	32.20 a	SD2	84.17 a	SD2	13.30 b	N0	37.80 b	N1	96.13 b	SD2	109.35 b						
	SD2N1	31.22 b					N1	41.38 A										

SD, sowing date; N, nitrogen; F, *Fusarium*; sedimentation test (SDSS); solvent retention capacities (sodium carbonate, SCRC; lactic acid, LARC; distilled water, WRC; sucrose, SuRC). *Means with the same letter are not significantly different between treatments ($p<0.05$, Duncan test).

~~*Means with the same letter are not significantly different between treatments ($p<0.05$, Duncan test).~~

Formatted: Line spacing: single

Table A.3. Effect of sowing date, nitrogen fertilization, and *Fusarium graminearum* on gliadin and glutenins subunits.

~~Percentage of the sum of squares respect to the model (SS%) and significance level of each source of variation (SV: SD, sowing date; N, nitrogen; F, *Fusarium*) for the ratio between the contents of: gliadins and glutenins (GLI/GLU), high molecular weight and low molecular weight glutenin subunits (HMW/LMW) and ω gliadins and α β γ gliadins (ω gli/α β γ gli), and the contents of different glutenin subunits encoded by *Glu-1* loci relative to total high molecular weight glutenins (Glu-A1x/HMW, Glu-B1x/HMW, Glu-B1y/HMW, Glu-D1x/HMW, Glu-D1y/HMW). Mean values were indicated for the significance SV with the highest level of a significant interaction.~~

Source of variations~~SV~~	GLI/GLU		HMW/LMW			ω-gli/α-β-γ-gli			GLU-A1x/HMW			GLU-B1x/HMW			GLU-B1y/HMW		GLU-D1x/HMW			GLU-D1y/HMW		
	SS%	p-value	SS%	p-value		SS%	p-value		SS%	p-value		SS%	p-value		SS%	p-value	SS%	p-value		SS%	p-value	
SD	26.0	0.2835	**22.0**	**0.0093**		**29.2**	**0.0391**		**44.7**	**0.0056**		**12.9**	**0.0092**		0.0	>0.9999	**30.0**	**0.0036**		**27.3**	**0.0216**	
N	1.7	0.6832	7.3	0.0619		23.0	0.0685		21.6	0.0728		20.0	0.1835		6.1	0.5471	3.4	0.1885		19.1	0.1296	
F	5.0	0.6329	2.5	0.3164		0.0	0.9566		11.1	0.1183		0.8	0.4536		13.6	0.2379	1.0	0.5167		3.0	0.3962	
SD*N	13.0	0.4431	**56.0**	**0.0003**		11.7	0.1692		7.1	0.2032		0.0	>0.9999		13.6	0.2379	**18.0**	**0.0159**		**19.1**	**0.0482**	
SD*F	8.5	0.5332	4.1	0.2074		1.3	0.6261		0.0	>0.9999		12.9	0.0092		6.1	0.4238	0.4	0.6955		12.3	0.1039	
N*F	0.3	0.9028	5.1	0.1603		0.1	0.8704		0.4	0.7423		7.1	0.0385		6.1	0.4238	0.0	0.8959		0.8	0.6678	
SD*N*F	10.2	0.4949	0.1	0.8254		2.8	0.484		1.8	0.5137		**12.9**	**0.0092**		13.6	0.2379	0.0	0.8959		6.8	0.2115	
Means	---		SD1N0	1.47	a	SD1	1.01	a	SD1	0.21	a	SD1N0F0	0.28	bc	---		SD1N0	0.22	a	SD1N0	0.16	a
												SD1N0F1	0.28	c								
			SD1N1	1.20	b							SD1N1F0	0.29	bc			SD1N1	0.20	b	SD1N1	0.17	a
												SD1N1F1	0.28	bc								
			SD2N0	1.15	b	SD2	0.91	b	SD2	0.19	b	SD2N0F0	0.29	b			SD2N0	0.23	a	SD2N0	0.16	ab

		SD2N1	1.28	b			SD2N0F1 SD2N1F0 SD2N1F1	0.28 0.29 0.30	bc b a		SD2N1	0.24	a	SD2N1	0.15	b			

SD, sowing date; N, nitrogen; F, *Fusarium*; GLI/GLU:) for the ratio between the contents of: gliadins and glutenins (GLI/GLU),; high molecular weight and low molecular weight glutenin subunits (HMW/LMW: high molecular weight and low molecular weight glutenin subunits;) and ω-gliadins and α-β-γ-gliadins (ω-gli/α-β-γ-gli: ω-gliadins and α-β-γ-gliadins), and the contents of different glutenin subunits encoded by *Glu-1* loci relative to total high molecular weight glutenins (Glu-A1x/HMW, Glu-B1x/HMW, Glu-B1y/HMW, Glu-D1x/HMW, Glu-D1y/HMW: the contents of different glutenin subunits encoded by *Glu-1* loci relative to total high molecular weight glutenins (). Mean values were indicated for the significance SV with the highest level of a significant interaction.*Means with the same letter are not significantly different between treatments ($p<0.05$, Duncan test).

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Line spacing: single

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman, 12 pt

Table A.4. Longevity parameters of accelerated aging assays ~~calculated as the area under the curve (viability, water content, and T50) for each treatment combination (SD, sowing date; N, nitrogen; F, *Fusarium*).~~

SD	N	F	Viability	Water content	T50
1	0	0	737.1	167.4	24
1	0	1	741.5	164.9	22.8
1	1	0	730.9	164.4	24.3
1	1	1	753.9	163.2	23.4
2	0	0	752.9	160.3	20.7
2	0	1	712.8	161.7	20.9
2	1	0	814.9	159.4	22.3
2	1	1	795.3	163.5	21.1

~~(viability, water content, and T50) for each treatment combination (~~SD, sowing date; N, nitrogen; F, *Fusarium*~~).~~

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Centered