Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
http://periodicos.ufersa.edu.br/index.php/sistema

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252015v28n301rc

# EFFECT OF *Fusarium graminearum* Schwabe ON PHYSIOLOGICAL QUALITY OF SOYBEAN SEEDS AND WHEAT CARYOPSIS IN ARGENTINA[1]

ALEJANDRA MARÍA PERUZZO[2]; ROSANNA NORA PIOLI[3]; ADRIANA RITA SALINAS[4]*

**ABSTRACT** - *F. graminearum* is the main causal agent of Head blight in cereals in Argentina. This is a disease that develops during the host floral state. When the reproductive structures in the host are invaded, grains may be shriveled and reduced in weight, causing a decrease in yield. Physiological diagnostic techniques on seeds detect the damages produced by this fungus could be used to take decisions related to the quality of seed lots. The objective of this study was to evaluate the possible physiological damage caused by *F. graminearum* isolates in soybean seeds and wheat caryopsis. Seeds and caryopsis were obtained from plants exposed to fungal infection and were evaluated under two situations: artificial inoculations under greenhouse conditions and natural infection from fields of Santa Fe Province (33°43'22''S; 62°14'46''W). Seed weight, topographical tetrazolium test, standard germination test, electrical conductivity test and X-ray test were performed in soybean seeds and wheat caryopsis from each treatment. Differential behaviors of *F. graminearum* strains in susceptible soybean and wheat cultivars under greenhouse conditions revealed specific interactions among soybean and wheat genotypes with this fungus. *F. graminearum* infection in susceptible cultivars under greenhouse conditions produced a significant decrease in the physiological quality of soybean seed and wheat caryopsis. These behaviors were not detected under field conditions in the evaluated locations. All seed quality tests used in this experiment were useful to show differences in infection in soybean and wheat independently of *F. graminearum* infection.

**Keywords**: X-Ray. Tetrazolium. Germination. Electric conductivity. Pampean region.

## EFEITO DE *Fusarium graminearum* Schwabe NA QUALIDADE FISIOLÓGICA DE SEMENTES DE SOJA E CARIOPSE DE TRIGO NA ARGENTINA

**RESUMO -** *F. graminearum* é o principal agente causal da giberela em cereais na Argentina. É uma doença que se desenvolve durante o estado floral de hospedeiro. Quando as estruturas reprodutivas no hospedeiro são colonizadas, os grãos podem ser enrugados e mostrar reduções de peso, causando diminuição no rendimento. Técnicas de diagnóstico fisiológico em sementes podem detectar os danos produzidos por este fungo e pode ser usada para tomar decisões sobre a qualidade dos lotes. O objetivo deste estudo foi avaliar possíveis danos fisiológicos causados por *F. graminearum* em sementes de soja e cariopses de trigo. As sementes e cariopses foram obtidas a partir de plantas expostas a infecção fúngica e foram avaliadas em duas situações: inoculação artificial em casa de vegetação e infecção natural em campos da Província de Santa Fé (33°43'22''S; 62°14'46''O). Determinou-se o peso da semente e realizou-se os testes de tetrazólio, germinação, condutividade elétrica e raios-X em sementes de soja e cariopses de trigo para cada tratamento. Comportamentos diferenciais de cepas de *F. graminearum* em cultivares suscetíveis de soja e trigo sob condições de casa de vegetação revelou interações específicas entre cultivares de soja e trigo com este fungo. As interações produziram uma redução significativa na qualidade fisiológica de sementes de soja e cariopses trigo. Esses comportamentos não foram detectados em condições de campo nos locais avaliados. Todos os testes de qualidade das sementes utilizados neste experimento foram úteis para mostrar as diferenças de infecção em sementes de soja e cariopses de trigo, independentemente da infecção por *F. graminearum*.

**Palavras-chave**: Teste de raios-X. Tetrazólio. Germinação. Condutividade elétrica. Região pampeana.

*Corresponding author
[1]Received for publication in 23/10/2014; accepted on 20/03/2015.
M.Sc. A thesis in Natural Resources of the first author.
[2]CONICET, Vegetal and Microbial Biodiversity Laboratory, Fac. Cs. Agrarias (Faculty of Agricultural Sciences), UNR (National University of Rosario), CC 14, S2125 ZAA, Zavalla, Santa Fe, Argentina; alejandra.peruzzo@unr.edu.ar.
[3]CIUNR (National University of Rosario Research Council), Responsible of Vegetal and Microbial Biodiversity Laboratory, Fac. Cs. Agrarias, UNR, CC 14, S2125 ZAA, Zavalla, Santa Fe, Argentina; rpioli@unr.edu.ar.
[4]CIUNR (National University of Rosario Research Council), Fac. Cs. Agrarias, UNR, Rosario, Santa Fe, Argentina; arsalinas@gmail.com.

## INTRODUCTION

*Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch] is the main causal agent of Head blight in cereals in Argentina (RAMIREZ et al., 2006). Epidemics registered in Argentinean Pampean Region were reported in 1978, 1985, 1993, 2001, 2007 and 2012 (MOSCHINI et al., 2001; LORI et al., 2003; VELAZQUEZ; FORMENTO, 2013). The disease progresses under warm and humid conditions, especially with a temperature between 20 to 30 °C. This disease is a monocyclic one, which develops principally in host floral state. Initially, the fungus grows its parasitic state over functional tissues, and later the saprophytic state over death tissues (crop residues). For this reason, the inoculum is available all year, which generates the possibility of new infections in cereals and other novel hosts (PIOLI et al., 2004; SCHAAFSMA et al., 2005).

*F. graminearum* produces a pink, orange to red discolouration on the glumes and premature death of upper spikes of the ear above the initial point of infection by obstruction of rachis vascular tissue (LORI et al., 2003; LEWANDOWSKI et al., 2006). When the ears are invaded in very early stage, grains may be shriveled and reduced in weight due to a decrease in water and nutrient levels (BROWN et al., 2010). The development of fungal hyphae produces simultaneously cytoplasm and organelle degradation in parenchyma plant cells (WANJIRU et al., 2002). Also the cellular wall damage of the grains caused by degradative fungal enzymes and mycotoxin production reduce the yields (ZHOU et al., 2005). As a result, raw materials and their commodities are rejected from imported countries (LAZZARI, 2000).

Physiological diagnostic techniques on seeds detect the damages produced by this fungus (ARGYRIS et al., 2003; YANG et al., 2012); and this information could be used to take decisions regarding the value of seed lots (ISTA, 2012). Among seed international tests, the X-ray analysis is a direct and innovative method that allows to measure seed quality without effect on seed viability (BELIN et al., 2011, SALINAS et al., 2012). It is non-destructive and quick method that enables to recognize the internal structure of the seed, and by default, detect hyphae presence (SALINAS et al, 2009, SALINAS et al., 2010). The electrical conductivity test is an indirect method that recognizes those seed characteristics associated with vigor and seedling performance (ISTA, 2012). As a consequence, this technique enables to characterize seed lots through the differences in electrolytic lixiviates. High values of electrolytic lixiviation and electric conductivity are associated with a decreased seed quality due to degradation membranes. The conductivity is measured continuously, providing a better analysis of this test (SALINAS et al., 2010). Likewise, some classical physiological seed tests such as topographic biochemical tetrazolium and standard germination tests were performed complementally to those novel techniques applied in this study (ISTA, 2012).

The objective of this study was to evaluate possible physiological damage caused by *F. graminearum* isolates in soybean seeds and wheat caryopsis.

## MATERIALS AND METHODS

The capacity of *F. graminearum* to reduce the physiological quality of soybean seeds (*Glycine max* (L.) Merr.) and wheat caryopsis (*Tritichum aestivum* L.) obtained from plants exposed to fungal infection was evaluated under different conditions: i) with artificial inoculations under greenhouse conditions, and ii) with natural infection from fields of Santa Fe Province. In greenhouse experiment, soybean seeds of susceptible cultivar CSOSU.1 (PIOLI et al., 2004) and caryopsis from two susceptible wheat cultivars, Federal (MOSCHINI et al., 2001) and BioINTA 1006 (VELAZQUEZ; FORMENTO, 2013), were manually collected with their respective experimental greenhouse control non-inoculated (PERUZZO et al., 2012). On the other hand, the same cultivars were collected from the field under natural conductive conditions for *F. graminearum*. Besides, other soybean and wheat cultivars were incorporated to evaluate different environments (Tables 1 and 2).

**Table 1**. Soybean seeds samples from plants exposed to *Fusarium graminearum* infection in artificial and natural environments.

| Environmental Condition | Cultivar | Origin, recollection year |
|---|---|---|
| i. Inoculation in greenhouse | CSOSU.1 (Fg1) | Zavalla, 2012 |
| | CSOSU.1 (Fg2) | Zavalla, 2012 |
| | CSOSU.1 (Fg3) | Zavalla, 2012 |
| | CSOSU.1 (FC) | Zavalla, 2012 |
| ii. Crop fields | CSOSU.1 | Venado Tuerto, 2010 |
| | A3700 | Salto, 2010 |
| | Torcacita 58 | Salto, 2010 |
| | NA3731 | Salto, 2010 |

[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

**Table 2**. Wheat caryopsis samples from plants exposed to *Fusarium graminearum* infection in artificial and natural environments.

| Environmental condition | Cultivar | Origin, recollection year |
|---|---|---|
| i. Inoculation in greenhouse | Federal (Fg1) | Zavalla, 2012 |
| | Federal (Fg2) | Zavalla, 2012 |
| | Federal (Fg3) | Zavalla, 2012 |
| | Federal (FC) | Zavalla, 2012 |
| | BioINTA (Fg1) | Zavalla, 2012 |
| | BioINTA (Fg2) | Zavalla, 2012 |
| | BioINTA (Fg3) | Zavalla, 2012 |
| | BioINTA (FC) | Zavalla, 2012 |
| ii. Crop fields | Federal | Oliveros, 2010 |
| | BioINTA | Oliveros, 2010 |
| | Gavilán | Pujato, 2010 |
| | Cronox | Pujato, 2010 |
| | Baguette 9 | Casilda, 2010 |
| | Baguette 11 | Fuentes, 2010 |
| | Baguette 17 | Casilda, 2010 |
| | ACA315 | Casilda, 2010 |
| | Themix 1 | Casilda, 2010 |
| | Themix 2 | Casilda, 2010 |

[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

After collection, all samples were stored in brown paper bags under cool conditions (4 °C). Due to the moisture of seeds and caryopsis need to be within 10 -14% to perform physiological tests (ISTA, 2012), water content was measured by two methods. When 100 g of seeds or caryopsis was available, moisture measurement equipment was used (MOTOMCO, Model 999-fr, Argentina). If it was no possible to be used, the moisture determination was made by the constant-temperature oven method (ISTA, 2012). The procedure was carried out in duplicate on two independently drawn working samples, each of 5 g using containers with 5 cm of diameter. Conditioning and distribution of samples into the containers were made according to ISTA (2012). Moisture content as a percentage (M%) was calculated with three decimals for each replicate by the following formula: M%=[(M2 -M3) / (M2 -M1)] *100, where M1 was the weight in grams of the container and its cover; M2 was the weight in grams of the container, its cover and its contents before drying; and M3 was the weight in grams of the container, its cover and its content after drying. The maximum difference for each sample between two replicates did not exceed 0.2% (ISTA, 2012).

Seed size was determined by weighing 100 randomly selected soybean seeds and wheat caryopsis from each treatment. The procedure was performed with an analytical laboratory balance with four numbers of decimal places.

Four replicates of 50 soybean seeds or wheat caryopsis were used for the topographical tetrazolium test (CRAVIOTTO; ARANGO PEREARNEAU; GALLO, 2008). Conditioning of seeds and caryopsis were made according to AOSA (1992). They were imbibed on moist rolled paper towels overnight. Seeds and caryopsis were placed in wells and covered with a 0.1% (soybean) or 0.5% (wheat) solution of 2, 3, 5-Triphenyl Tetrazolium Chloride (Cecarrelli) for three hours in darkness. Results were expressed as a percentage of viable soybean seeds or wheat caryopsis.

Standard germination tests were conducted in four replicates of 50 soybean seeds and wheat caryopsis according to the between-paper method (ISTA, 2012). Fifty seeds or caryopsis per four repetitions were placed equidistant over two moist germination paper (Anchor Paper 54×30cm, Agricol (Pty) Ltd, South Africa), which were rolled up and placed in polyethylene bags. These were sealed and incubated in an upright position at 25 °C for soybean and 20 °C for wheat. Percentage of germination was determined after eight days using the Seedling Evaluation Handbook (AOSA, 1992).

Electrical conductivity was measured to 100 seeds or caryopsis individually during twenty hours of immersion at intervals of three minutes. The assay was performed using the Automatic Analyzer SAD 9000-S (MR Consultar Ingeniería Informática), Rosario, Argentina. Each seed or caryopsis was placed in a plastic well containing seven mL of sterile distilled water (conductivity between 0 and five $\mu S.cm^{-1}$). The group of 100 seeds or caryopsis from each treatment was weighted prior to immersion, and the results of the electric conductivity measurement were expressed in $\mu S.cm^{-1}.g^{-1}$.

SEMAX (INTA-TEXEL-FCA, Argentina) X-ray equipment was used, which permitted a non-destructive method for seed analysis. This equipment also complies with the safety regulations demanded by sanitary radiophysics using the latest techniques of digital radiographic images to a 35 kW, an intensity of 10 mA and an exposition time of 0.65 s. The system for capturing and digitalizing images is Visualix (2000). Digital radiographic images were processed, manipulated, measured and stored. A hundred soybean seeds and wheat caryopses were evaluated per treatment, in which three soybean seeds or five caryopses of wheat were placed per cell in a sample holder reel. Evaluation of the radiographic image to score fungi presence was made by direct observation of digital images and its corresponding photographical images.

Seed size treatments were subjected to hierarchical agglomerative cluster analysis to estimate Jaccard's Similarity Coefficient and Ward's minimum variance method as the clustering algorithm. Data from topographical tetrazolium, standard germination, and X-ray tests were subjected to analysis of variance using Duncan's multiple range test ($P<0.05$). All tests were analyzed by Infostat program (Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina). Electrical Conductivity behavior over time was performed through Graph Pad Prism Programme (La Jolla, CA, USA), according to the previous election of the statistical model (SALINAS et al., 2010). Treatments were characterized and clustered by Principal Component Analysis of the two main parameters of the curves.

## RESULTS AND DISCUSSION

Seeds and caryopsis moisture evaluation were consistent with what was expected for ISTA (2012) in all samples. Weight rates for soybean seeds and wheat caryopsis are shown in Tables 3 and 4, including cultivars exposed to artificial and natural interaction with *F. graminearum*.

**Table 3**. Weight for soybean seeds from plants exposed to artificial and natural infection with *Fusarium graminearum*.

| Environmental condition | Cultivar | Peso (g)* |
|---|---|---|
| i. Inoculation in greenhouse | CSOSU.1 (Fg1) | 89,654 a |
| | CSOSU.1 (Fg2) | 89,825 a |
| | CSOSU.1 (Fg3) | 96,872 a |
| | CSOSU.1 (FC) | 92,511 a |
| ii. Crop fields | CSOSU.1 | 93,801 a |
| | A3700 | 171,402 b |
| | Torcacita58 | 150,379 b |
| | NA3731 | 130,750 b |

*Values with the different minus letter are statistical significantly different.
[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

Seed weight of susceptible soybean from artificial or natural infection, were greatly reduced (between 31 to 47%) about commercial cultivars (Table 3). Among *F. graminearum* - CSOSU.1 interactions, Fg1, and Fg2 isolates were more virulent, decreasing 10% seed weight with respect to Fg3 and Control.

**Table 4**. Weight for wheat caryopsis from plants exposed to artificial and natural infection with *Fusarium graminearum*.

| Environmental condition | Cultivar | Peso (g)* |
|---|---|---|
| i. Inoculation in greenhouse | Federal (Fg1) | 24,730 a |
| | Federal (Fg2) | 28,710 a |
| | Federal (Fg3) | 24,840 a |
| | Federal (FC) | 33,364 b |
| | BioINTA(Fg1) | 27,606 a |
| | BioINTA(Fg2) | 25,856 a |
| | BioINTA(Fg3) | 26,202 a |
| | BioINTA(FC) | 20,614 a |
| ii. Crop fields | Federal | 17,729 a |
| | BioINTA | 37,433 b |
| | Gavilán | 31,051 a |
| | Cronox | 37,188 b |
| | Baguette9 | 37,436 b |
| | Baguette11 | 35,364 b |
| | Baguette17 | 30,232 a |
| | ACA315 | 37,918 b |
| | Themix1 | 29,840 a |
| | Themix2 | 30,314 a |

*Values with the different minus letter are statistical significantly different.
[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

Federal susceptible cultivar showed the lowest weight of seeds as in greenhouse as in natural field conditions (Table 4). Also seed weight from inoculated plants by Fg1, Fg2 and Fg3 was almost 21% lower than its control in the greenhouse. With respect to the susceptible BioINTA cultivar, the weight of seeds from control and inoculated plants in the greenhouse was lower than those produced under field conditions (BioINTA, Cronox, Baguette 9 and 11, ACA315). However, seed weight from control was lower than those inoculated plants, indicating some particular biotic and abiotic stress (Table 4). Exposition to different field environments could explain the variable behavior of different cultivars under natural conditions.

Viability determination by tetrazolium test showed statistical differences between treatments, being soybean CSOSU.1. One no inoculated and A3700 those who showed the best viability. The lowest viability was seen in soybean CSOSU.1 inoculated treatments (Table 5).

**Table 5**. Seed Viability (%) by topographical tetrazolium Test for cultivars exposed natural and force interactions with *Fusarium graminearum*.

| Environmental condition | Cultivar | Viability (%)* |
|---|---|---|
| i. Inoculation in greenhouse | CSOSU.1 (Fg1) | 68 a |
| | CSOSU.1 (Fg2) | 68 a |
| | CSOSU.1 (Fg3) | 65 a |
| | CSOSU.1 (FC) | 82 b |
| ii. Crop fields | CSOSU.1 | 97 c |
| | Torcacita58 | 90 bc |
| | A3700 | 97 c |
| | NA3731 | 89 b |

*Values with the different minus letter are statistical significantly different.
[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

Seed viability of susceptible soybean showed significant differences between those from plants inoculated with *F. graminearum* and both controls, from greenhouse and natural field conditions, according to Govender; Aveling; Kritzinger (2008). Plants inoculated with Fg1; Fg2 and Fg3 isolate produced seeds with the lowest viability values (Table 5). These results were consistent with weight determination (Table 4). Moreover, seeds obtained from four cultivars under natural field conditions (susceptible CSOSU.1 and three commercial cultivars), showed good viability; pointing to field environmental conditions were not conductive for the *F. graminearum* infection.

Viability by topographical tetrazolium test in wheat cultivars is showed in Table 6.

**Table 6**. Seed viability (%) by topographical tetrazolium test for cultivars exposed natural and force interactions with *Fusarium graminearum.*

| Environmental condition | Cultivar | Viability (%)* |
|---|---|---|
| i. Inoculation in greenhouse | Federal (Fg1) | 90 cde |
| | Federal (Fg2) | 77 ab |
| | Federal (Fg3) | 86 bc |
| | Federal (FC) | 88 cde |
| | BioINTA (Fg1) | 92 cdef |
| | BioINTA (Fg2) | 73 a |
| | BioINTA (Fg3) | 86 bc |
| | BioINTA (FC) | 99 i |
| ii. Crop fields | Federal | 98 hi |
| | BioINTA | 96 fgh |
| | Gavilán | 98 ghi |
| | Cronox | 94 cdef |
| | Baguette9 | 96 fgh |
| | Baguette11 | 95 efg |
| | Baguette17 | 95 defg |
| | ACA315 | 88 cd |
| | Themix1 | 97 efgi |
| | Themix2 | 97 fgh |

*Values with the different minus letter are statistical significantly different.
[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

Seeds of susceptible wheat cultivars (Federal and BioINTA) produced from inoculated plants with three F. *graminearum* isolates showed viability value lower than those obtained from six cultivars exposed to field conditions, except ACA315, putting in evidence the fungal damage. However, the effect on the viability was different depending on the fugal isolate. So, seeds from Federal and BioINTA inoculated with Fg2 showed the lowest viability. Seeds from BioINTA control showed high viability while Federal control showed lower viability that seeds from inoculated plants, possibly due to some biotic factor (insects). On the other hand, both susceptible wheat cultivars exposed to natural field condition showed good performance, similarly to caryopsis viability from commercial cultivars.

Standard germination test for soybean cultivars belonging to susceptible and commercial cultivars is shown in Table 7.

**Table 7.** Standard germination (%) for soybean seeds at 25°C.

| Environmental condition | Cultivar | Germination (%)* |
|---|---|---|
| i. Inoculation in greenhouse | CSOSU.1 (Fg1) | 36 a |
| | CSOSU.1 (Fg2) | 58 b |
| | CSOSU.1 (Fg3) | 47 ab |
| | CSOSU.1 (FC) | 44 ab |
| ii. Crop fields | CSOSU.1 | 96 c |
| | Torcacita58 | 97 c |
| | A3700 | 100 d |
| | NA3731 | 98 cd |

*Values with the different minus letter are statistical significantly different.
[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

Seed germination of CSOSU.1 cultivar was severely affected when plants were inoculated with three *F. graminearum* (Fg2, Fg3 and mainly Fg1, Table 7). However, CSOSU.1 used as greenhouse control presented moderate performance with respect to CSOSU.1 and others commercial cultivars exposed to field conditions. Germination, viability and weight of seeds of soybean susceptible cultivar (CSOSU.1) have put in evidence the physiological damage caused by *F. graminearum*.

In wheat caryopsis, the results of the standard germination Test is exposed in Table 8.

**Table 8**. Standard germination (%) for wheat caryopsis at 20 °C.

| Environmental condition | Cultivar | Germination (%)* |
|---|---|---|
| i. Inoculation in greenhouse | Federal (Fg1) | 98 fg |
| | Federal (Fg2) | 85 abc |
| | Federal (Fg3) | 96 def |
| | Federal (FC) | 86 abc |
| | BioINTA (Fg1) | 84 abc |
| | BioINTA (Fg2) | 76 a |
| | BioINTA (Fg3) | 94 def |
| | BioINTA (FC) | 99 g |
| ii. Crop fields | Federal | 96 def |
| | BioINTA | 99 g |
| | Gavilán | 86 abc |
| | Cronox | 93 cde |
| | Baguette9 | 90 bcd |
| | Baguette11 | 80 a |
| | Baguette17 | 84 abc |
| | ACA315 | 81 ab |
| | Themix1 | 96efg |
| | Themix2 | 94 de |

*Values with the different minus letter are statistical significantly different.
[Fg1]Isolate from the pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

This test showed differences between cultivars. The lowest levels of germination corresponded to BioINTA inoculated by Fg1, and Fg2, Federal cultivar exposed to Fg2, and Federal cultivar used as greenhouse control. These results reveal the damages caused by biotic (fungi) and abiotic stress in the greenhouse, respectively (Table 8). Six wheat seed samples (both wheat susceptible and four cultivars) exposed to natural field conditions showed the best germination rates. However, Gavilán, Baguette 11, Baguette 17 and ACA 315 cultivars, showed low germination values possibly due to adverse field environmental conditions or storage problems after harvest, as was previously reported by da Rosa et al. (2011).

The results of the seed vigor by electrical conductivity test showed differences between treatments. In susceptible and commercial cultivars in the soybean seeds, the results are showed in Figure 1.

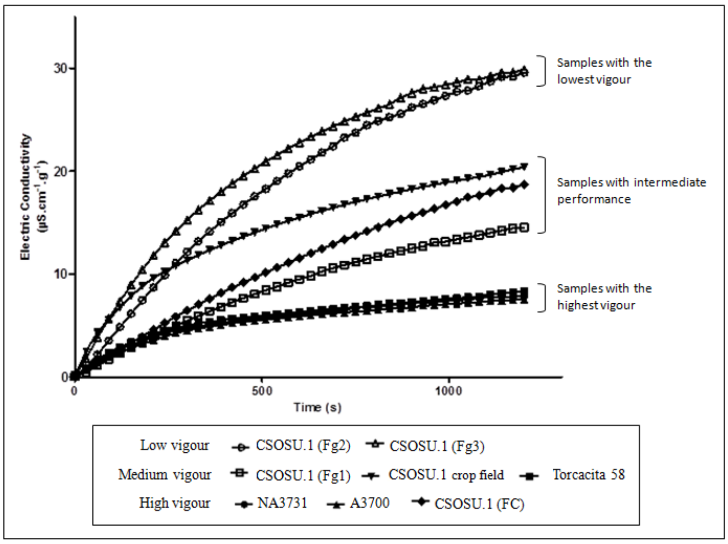


**Figure 1**. Electric conductivity measurement (µS.cm$^{-1}$.g$^{-1}$) of the soybean treatments during twenty hours.

This test revealed that cultivar CSOSU.1 inoculated with Fg2, and Fg3 showed the highest electrical conductivity, meaning the lowest vigor. The highest vigor was seen in all commercial cultivars exposed to natural infection in the field: Torcacita 58, A3700 and NA3731. The other treatments showed intermediate performances. In conclusion, these results agreed what was previously obtained by other tests.

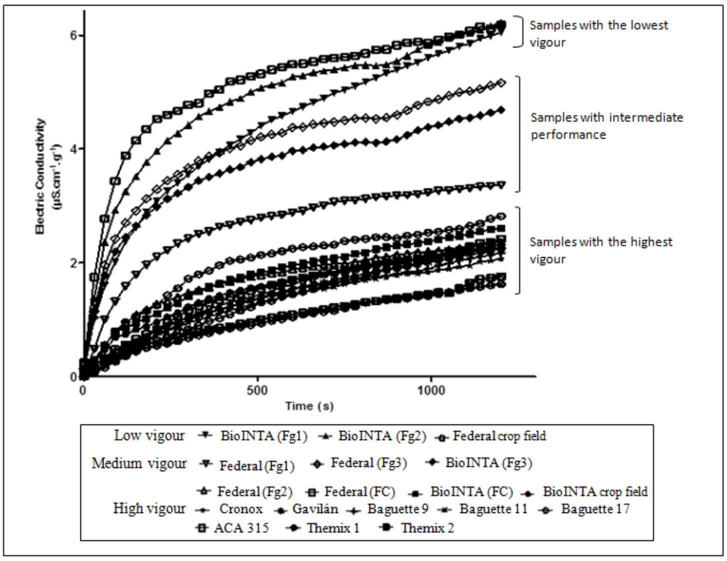


**Figure 2**. Electric conductivity measurement (µS.cm$^{-1}$.g$^{-1}$) of the wheat treatments during twenty hours.

The highest electrical conductivity and the lowest of vigor values were registered in BioINTA wheat caryopsis inoculated with both Fg1, and Fg2, and Federal caryopsis exposed to natural field conditions (Figure 2). These results revealed damages caused by biotic (fungi) stress in the greenhouse, and abiotic stress in field conditions (Table 8). The highest vigour was seen in Federal and BioINTA control of greenhouse, nine cultivars exposed to field conditions (BioINTA, ACA 315, Gávilan, Cronox, Baguette 9, Baguette 11, Baguette 17, Themix 1 and Themix 2), and Federal cultivar inoculated with Fg2. The other treatments showed intermediate performances. These results allow recognize the electrical conductivity test as a good technique to measure seed quality, contrary to previous reports about *Phomopsis* and *F. graminearum* associated to seeds (ZORRILLA et al., 1994; ARGYRIS et al., 2003)

Radiographic images allowed us to digitally characterized soybean seeds and wheat caryopsis. Also, X-ray images were compared with photographic images to evaluate mycelia presence (Figures 3 and 4).

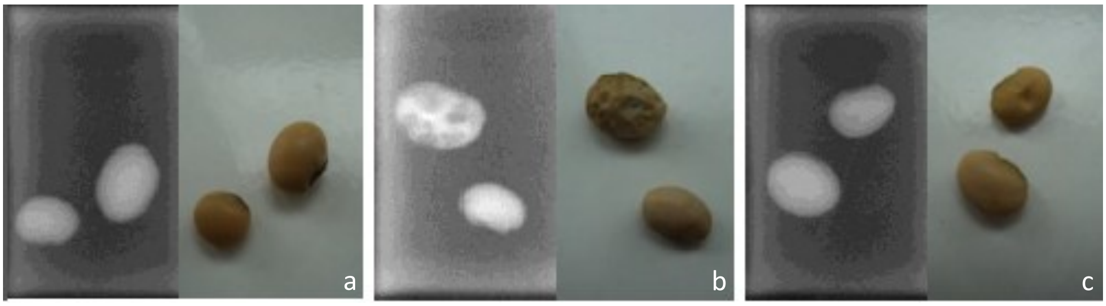


**Figure 3**. Digital radiographic and photographic images of soybean seeds. **a.** Filled seeds; **b.** Radiographic and photographic images of seed folds produced by environmental damage (above); **c.** Radiographic and photographic images of seed with bug damage (above).

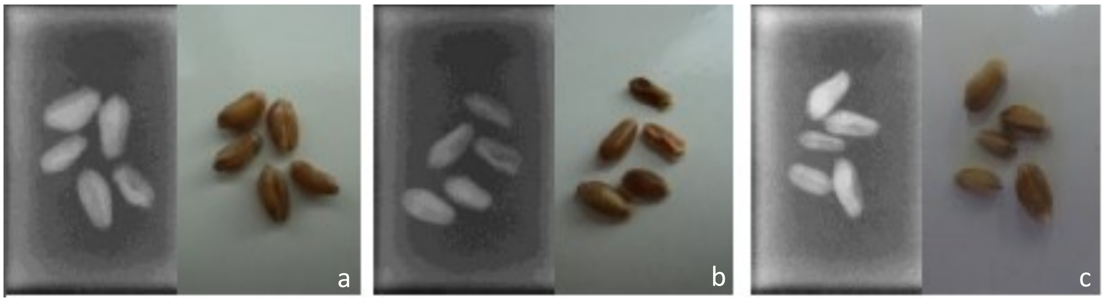


**Figure 4**. Digital radiographic and photographic images of wheat caryopsis. **a.** Filled caryopsis; **b.** Radiographic and photographic abnormal caryopsis (above); **c.** Radiographic and photographic caryopsis with the development of fungal mycelium (second above).

X-Ray Test was negative for fungus mycelia presence in all soybean and wheat samples, except in the BioINTA wheat cultivar inoculated with Fg1 (Tables 9 and 10).

**Table 9**. Soybean seeds samples characterized (%) by X-ray test.

| Environmental Condition | Cultivar | Seeds (%) | | |
|---|---|---|---|---|
| | | Filled | Empty | Other |
| i. Inoculation in greenhouse | CSOSU.1 (Fg1) | 90 | | 4 (insect); 6 (ambiental damage) |
| | CSOSU.1 (Fg2) | 78 | 1 | 19 (insect); 2(ambiental damage) |
| | CSOSU.1 (Fg3) | 65 | | 14 (insect); 21(ambiental damage) |
| | CSOSU.1 (FC) | 75 | | 10 (insect); 15(ambiental damage) |
| ii. Crop fields | CSOSU.1 | 100 | | |
| | A3700 | 100 | | |
| | Torcacita 58 | 100 | | |
| | NA3731 | 100 | | |

[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from soybean, [FC]Greenhouse control.

Considering that the test was negative for fungal mycelium for all the soybean samples (Table 9), it is possible to infer that low percentage of filled seeds from inoculated (CSOSU.1-Fg2 and CSOSU.1 -Fg3), and control (CSOSU.1-FC) under greenhouse conditions were caused by insects and abiotic stress. Although the CSOSU.1 inoculated by Fg1 obtained 90% of filled seeds. It is interesting to point out that results about inoculated CSOSU.1 (Figures 1 and 3), in greenhouse were coherent with the significant low weights (Table 3) and moderate, and low vigor registered through electrical conductivity test (Figure 1).

While all seed samples from commercial cultivars exposed to different location or field environments showed 100% filled seeds (Table 9) with the highest weights (Table 3), and moderate and high vigor defined by electrical conductivity (Figure 1); meaning that the field conditions were no conductive for the interaction soybean cultivars and *F. graminearum*.

**Table 10**. Wheat caryopses samples characterized (%) by the X-ray test.

| Environmental condition | Cultivar | Caryopsis (%) | | |
|---|---|---|---|---|
| | | filled | empty | other |
| i. Inoculation in greenhouse | Federal (Fg1) | 92 | 8 | |
| | Federal (Fg2) | 98 | 2 | |
| | Federal (Fg3) | 80 | 20 | |
| | Federal (FC) | 97 | 3 | |
| | BioINTA (Fg1) | 83 | 16 | 1 (mycelium) |
| | BioINTA (Fg2) | 67 | 33 | |
| | BioINTA (Fg3) | 91 | 9 | |
| | BioINTA (FC) | 98 | 2 | |
| ii. Crop fields | Federal | 96 | 4 | |
| | BioINTA | 100 | | |
| | Gavilán | 100 | | |
| | Cronox | 99 | 1 | |
| | Baguette9 | 100 | | |
| | Baguette11 | 100 | | |
| | Baguette17 | 98 | 2 | |
| | ACA315 | 100 | | |
| | Themix1 | 99 | | 1 (physical) |
| | Themix2 | 100 | | |

[Fg1]Isolate from pea, [Fg2] Isolate from the bean, [Fg3] Isolate from the soybean, [FC]Greenhouse control.

Federal wheat inoculated with Fg3 registered a high number of empty caryopsis similarly to the BioINTA samples inoculated with Fg1, and Fg2 in the greenhouse. The reduction in quality would be associated with fungi interaction to compare with the behavior of respective greenhouse controls (Federal-FC and BioINTA-FC), but only one caryopsis sample (BioINTA-Fg1) evidenced presence of fungi mycelium. Therefore, the high percentage of empty caryopses obtained in greenhouse samples could due to another factor no registered by the test. These results are according to weight (Table 4) and vigor values (Figure 2).

On the other hand, all wheat samples (from two susceptible and commercial cultivars) obtained in natural field conditions registered a minimum value of empty caryopsis. And except Federal, they also showed high sanitary and physiologic quality according to weight and vigor registered (Table 4 and Figure 2, respectively). These results indicate that environmental conditions were non-conductive for *F. graminearum* infection. Regarding Federal showed a good performance by X-Ray, but registered low weight (Table 4) and vigor (Figure 2) similarly to those inoculated susceptible cultivars (BioINTA-Fg1 and BioINTA-Fg2), probably indicating a particular interaction with other abiotic or biotic factors non-detected by these tests.

Due to greenhouse controls showed good performances in different physiological tests, failures registered in inoculated soybean seeds and wheat caryopsis could be explained by fungal damage. This was supported by Argyris et al. (2003), who found out that infection by *F. graminearum* affected both the physical and the physiological aspects of seed quality, including seed size and weight, composition, and quality.

Since X-ray test did not show a significant presence of fungal mycelia, therefore physiological quality reduction by *F. graminearum* inoculation should be evaluated by sanitary seed Tests. Nevertheless, reduced quality was observed in Federal, and BioINTA samples inoculated with all the pathogens, related with high levels of empty caryopsis. Since *F. graminearum* mycelia was absent, damage could be explained by mycotoxin production during pathogenesis, since the soybean seeds, and the wheat caryopsis were evaluated for DON, and ZEA presence and resulted positive (PERUZZO et al, 2012). Meanwhile Del Ponte et al. (2007) y Boenisch, and Schäfer (2011) observed similar results in the same species reporting that mycotoxins levels upper than what is acceptable for the European Union, showed the lowest seed and caryopsis weight. These authors observed a significant negative correlation between kernel wheat weight and mycotoxin production. Besides, weight in the soybean seed or the wheat caryopsis also are supported by mycotoxins presence, as was previously reported by Kiekana et al. (2002), and Tekle et al. (2012). Additionally, *Fusarium* invasion could kill the germ and reduce seed germination, and yields. Such events were verified by different tests and are frequently associated with high levels of mycotoxins.

Although the soybean cultivar CSOSU.1, and BioINTA 1006 wheat are known as susceptible cultivars, the fungus was not found in field conditions. Even more, those treatments exhibited good performances in almost all the tests, as was observed for nearly all commercial cultivars despite the time difference. This demonstrates that if the fungus and host do not meet under conductive or favorable environment during a determined time, the disease did not progress.

Finally, this work shows important interactions between Argentinean *F. graminearum* isolates and two major worldwide crops: wheat and soybean; that express the necessity of continuous monitoring to preserve seed and food quality.

## CONCLUSIONS

Differential behaviors of *F. graminearum* strains in susceptible soybean and wheat cultivars under greenhouse conditions revealed specific interactions among the soybean and the wheat genotypes with this fungus.

*F. graminearum* infection in susceptible soybean and wheat cultivars under greenhouse conditions produced a significant decrease in the physiological quality of soybean seed and wheat caryopsis. These behaviors were not detected under field conditions in the evaluated locations.

All seed quality tests used in this experiment (tetrazolium test, standard germination test, electrical conductivity test and X-ray test) have been useful to show differences in soybean and wheat independently of *F. graminearum* infection.

## ACKNOWLEDGMENTS

This study was supported by the Faculty of Agricultural Sciences, Science & Technology Secretary and Research Council of Rosario National University.

## REFERENCES

ARGYRIS, J.; VAN SANFORD, D.; TEKRONY, D. Infection during Wheat Seed Development and Its Effect on Seed Quality. **Crop Science**, Madison, v. 43, n. 5, p. 1782-1788, 2003.

ASSOCIATION OF OFFICIAL SEED ANALYSIS. **Seedling evaluation handbook**. Washington (Columbia): Society of Commercial Seed Technologists, 1992. 102 p. (Contribution No. 35).

ASSOCIATION OF OFFICIAL SEED ANALYSIS. **Tetrazolium testing handbook**. Washington (Columbia): Society of Commercial Seed Technologists, 2000. 327 p. (Contribution No. 29).

BELIN, E. et al. Rate-distortion tradeoff to optimize high-throughput phenotyping systems. Application to X-ray images of seeds. **Computers and Electronics in Agriculture**, Linn, v. 77, n. 2, p. 188-194, 2011.

BOENISCH, M. J., SCHÄFER, W. *Fusarium graminearum* forms mycotoxin producing infection structures on wheat. **Plant Biology**, Malden, v. 11, p. 1-13, 2011.

BROWN, N. A. et al.The infection biology of *Fusarium graminearum*: Defining the pathways of spikelet to spikelet colonisation in wheat ears. **Fungal Biology**, Philadelphia, v. 114, n. 7, p. 555-571, 2010.

CRAVIOTTO, R.; ARANGO PEREARNEAU, M.; GALLO, C. **Prueba topográfica por tetrazolio en Soja**. Rosario (Rosario): Análisis de Semillas, 2008. 96 p. (Suplemento especial Nº 1).

DA ROSA, S. D. V. F. et al.The effect of storage conditions on coffee seed and seedling quality. **Seed Science and Technology**, Bassersdorf, v. 39, n. 1, p. 151-164, 2011.

DEL PONTE, E. M.; FERNANDES, J. M. C.; BERGSTROM, G. C. Influence of Growth Stage on Fusarium Head Blight and Deoxynivalenol Production in Wheat. **Journal of Phytopathology**, Malden, v. 155, n. 10, p. 577-581, 2007.

GOVENDER, V.; AVELING, T. A. S.; KRITZINGER, Q. The effect of traditional storage methods on germination and vigour of maize (*Zea mays* L.) from northern KwaZulu-Natal and southern Mozambique. **South Africa Journal of Botany**, Heidelberg, v. 74, n. 2, p. 190-196, 2008.

INTERNATIONAL SEED TESTING ASSOCIATION. **International Rules for Seed Testing**. Bassersdorf, Zwitzerland. International Seed Testing Association Work, 2012. 362 p.

KIECANA, I. et al. Scab response and moniliformin accumulation in kernels of oat genotypes inoculated with *Fusarium avenaceum* in Poland. **European Journal of Plant Pathology**, Heidelberg, v. 108, n. 3, p. 245–251, 2002.

LAZZARI, F. Rações e micotoxinas. In: SCUSSEL, V. M. (Ed.). **Atualidades em micotoxinas e armazenagem de grãos I**. Florianópolis: Universidade Federal de Santa Catarina, 2000. v. 1, cap. 5, p. 45-92.

LEWANDOWSKI, S. M., BUSHNELL, W. R., EVANS, C. K. Distribution of mycelial colonies and lesions in field-grown barley inoculated with *Fusarium graminearum*. **Phytopathology**, St. Paul, v. 96, n. 6, p. 567-581, 2006.

LORI, G. et al. *Fusarium graminearum* and deoxynivalenol contamination in the durum wheat area of Argentina. **Microbiological Research**, Linn, v. 158, n. 1, p. 29-35, 2003.

MOSCHINI, R.; CONTI, H.; CAZENAVE, G. **Estimación de la incidencia de *Fusariosis* en trigo,**

**Región pampeana. Campaña 2001**. Castelar: Instituto de Clima y Agua, 2001. s. p.

PERUZZO, A.; PIOLI, R.; SALINAS, A. Relación entre infección de *Fusarium graminearum* y transmisión de toxinas a las semillas y harinas de trigo y soja. In: CONGRESO SOCIEDAD DE BIOLOGIA DE ROSARIO, XIV., 2012, Casilda. **Anais XXXII**. Rosario: Asociación Civil Sociedad de Biología de Rosario, 2012. p. 166.

PIOLI R. N., MOZZONI L., MORANDI E. N. First Report on Pathogenic Association between *Fusarium graminearum* and Soybean. **Plant Disease**, St. Paul, v. 88, n. 2, p. 220, 2004.

RAMIREZ, M. L. et al.Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. **European Journal of Plant Pathology**, Heidelberg, v. 115, n. 2, p. 139-148, 2006.

SALINAS, A. et al. Caracterización de semillas de *Acacia longifolia* (Andr.) Willdenow a través de variables físicas y radiográficas. **Análisis de Semillas**, Rosario, v. 4, n. 12. p. 88-92, 2009.

SALINAS, A. et al. Caracterización radiográfica de semillas y frutos de especies forestales autóctonas y cultivadas. **Análisis de Semillas**, Rosario, v. 4, n. 16, p. 73-81, 2010.

SALINAS, A. et al. X-ray: Characterization of G*inkgo biloba* L. seeds using digital and manual measurements. **Revista Caatinga**, Mossoró, v. 25, n. 3, p. 1-7, 2012.

SCHAAFSMA, A. W., TAMBURIC-ILINCIC, L., HOOKER, D. C. Effect of previous crop, tillage, field size, adjacent crop, and sampling direction on airborne propagules of *Gibberella zeae*/*Fusarium graminearum*, fusarium head blight severity, and deoxynivalenol accumulation in winter wheat. **Canadian Journal of Plant Pathology**, Ottawa, v. 27, n. 2, p. 217-224, 2005.

TEKLE, S.; SKINNES, H.; BJØRNSTAD, Å. The germination problem of oat seed lots affected by Fusarium head blight. **European Journal of Plant Pathology**, Heidelberg, v. 135, n. 1, p. 147–158, 2012.

VELAZQUEZ, P. D., FORMENTO, A. N. Efecto de la fusariosis de la espiga del trigo (*Fusarium graminearum* y *Fusarium* spp.) en un cultivar susceptible. In: JORNADA REGIONAL DE CULTIVOS DE INVIERNO, Campaña 2013., 2013, Oro Verde. **Anais MMXIII**. Oro Verde: Universidad Nacional del Litoral, 2013. p. 45-48.

WANJIRU, W. M.; ZHENSHENG, K.; BUCHENAUER, H. Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat heads. **European Journal of Plant Pathology**, Heidelberg, v. 108, n. 8, p. 803-810, 2002.

YANG, F., SVENSSON, B., FINNIE, C. Response of germinating barley seeds to *Fusarium graminearum*: The first molecular insight into *Fusarium* seedling blight. **Plant Physiology and Biochemistry**, Heidelberg, v. 49, n.11, p. 1362-1368, 2011.

ZHOU, W., KOLB, F. L., RIECHERS, D. E. Identification of proteins induced or upregulated by *Fusarium* head blight infection in the spikes of hexaploid wheat (*Triticum aestivum*). **Genome**, Ottawa, v. 48, n. 5, p. 770-780, 2005.

ZORRILLA, G.; KNAPP, A. D.; MCGEE, D. C. Severity of *Phomopsis* Seed Decay, Seed Quality Evaluation, and Field Performance of Soybean. **Crop Science**, Madison, v. 34, n. 1, p. 172-177, 1994.