



**HAL**  
open science

## Priming to protect maize from *Fusarium verticillioides* and its fumonisin accumulation

Ana Aguado, Jean-Michel Savoie, Sylvain Chéreau, Christine Ducos, Maria Aguilar, Nathalie Ferrer, Manuel Aguilar, Laetitia Pinson-Gadais, Florence Richard-Forget

► **To cite this version:**

Ana Aguado, Jean-Michel Savoie, Sylvain Chéreau, Christine Ducos, Maria Aguilar, et al.. Priming to protect maize from *Fusarium verticillioides* and its fumonisin accumulation. *Journal of the Science of Food and Agriculture*, 2019, 99 (1), pp.64-72. 10.1002/jsfa.9142 . hal-02155923

**HAL Id: hal-02155923**

**<https://hal.science/hal-02155923v1>**

Submitted on 14 Jun 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Priming to protect maize from *Fusarium verticillioides* and its fumonisin accumulation

Journal:	<i>Journal of the Science of Food and Agriculture</i>
Manuscript ID	JSFA-18-0077.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	26-Apr-2018
Complete List of Authors:	<p>Aguado, Ana; Instituto Andaluz de Investigacion y Formacion Agraria Pesquera Alimentaria y de la Produccion Ecologica, Sustainable Plant Protection</p> <p>Savoie, Jean-Michel; MycSA, UR1264, INRA, 71 Avenue Edouard Bourleaux, F-33883 Villenave d'Ornon Cedex, France, MycSA</p> <p>Chereau, Sylvain; UR1264, INRA, 71 Avenue Edouard Bourleaux, F-33883 Villenave d'Ornon Cedex, France, MycSA,</p> <p>Ducos, Christine; UR1264, INRA, 71 Avenue Edouard Bourleaux, F-33883 Villenave d'Ornon Cedex, France, MycSA</p> <p>Aguilar, Maria; Instituto Andaluz de Investigacion y Formacion Agraria Pesquera Alimentaria y de la Produccion Ecologica, Crop Protection</p> <p>Ferrer, Nathalie; UR1264, INRA, 71 Avenue Edouard Bourleaux, F-33883 Villenave d'Ornon Cedex, France, MycSA</p> <p>Aguilar, Manuel; Instituto Andaluz de Investigacion y Formacion Agraria Pesquera Alimentaria y de la Produccion Ecologica, Agriculture and Environment</p> <p>Pinson-Gadais, Laetitia; UR1264, INRA, 71 Avenue Edouard Bourleaux, F-33883 Villenave d'Ornon Cedex, France, MycSA</p> <p>Forget, Florence; UR1264, INRA, 71 Avenue Edouard Bourleaux, F-33883 Villenave d'Ornon Cedex, France, MycSA</p>
Key Words:	priming, germination, mycotoxins, Fusarium disease, maize

1 ***Priming to protect maize from *Fusarium verticillioides* and its fumonisin***  
2  
3 ***accumulation***  
4  
5

6  
7 **ABSTRACT**  
8

9 **BACKGROUND**  
10

11 Systemic infection through the seed is one of the routes used by mycotoxinogen pathogen  
12 *Fusarium verticillioides* for colonizing maize plants. The prohibition of most of the chemical  
13 fungicides by the EU promote the research of plant resistance inducers as an effective and  
14 sustainable alternative. Induction of a priming state in maize seeds might affect their  
15 susceptibility for contamination and accumulation of fumonisins. This state, obtained by application  
16 of a natural fertilizer called Chamae on maize seeds, was investigate in two varieties to  
17 control the colonization by the fungus and the accumulation of fumonisins B<sub>1</sub>, B<sub>2</sub>, and  
18 B<sub>3</sub>.  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29

30 **RESULTS**  
31

32  
33 After inoculation of *F. verticillioides* on germinating seeds, the colonization by the fungus  
34 and the accumulation of fumonisins were significantly lower in seedlings coming from  
35 treated seeds, but a significant number of plant stop their development by necrosis. In a field  
36 trial, the 0,01% application dilution showed a lower plant density although the level of  
37 biomass at harvest was not affected.  
38  
39  
40  
41  
42  
43  
44

45 **CONCLUSION**  
46

47  
48 The priming state contributed to the control of *F. verticillioides* development from seed  
49 infection and fumonisin accumulation in the early stage of plant growth, without affecting the final  
50 crop yield and could  
51 reduce the fungicide use and environmental contamination.  
52  
53  
54  
55  
56  
57  
58  
59  
60

## **Priming to protect maize from *Fusarium verticillioides* and its fumonisin accumulation**

Ana Aguado<sup>a\*</sup>, Jean-Michel Savoie<sup>b\*</sup>, Sylvain Chéreau<sup>b</sup>, Christine Ducos<sup>b</sup>, María Aguilar<sup>a</sup> Nathalie Ferrer<sup>b</sup>, Manuel Aguilar<sup>a</sup>, Laetitia Pinson-Gadais<sup>b</sup>, Florence Richard-Forget<sup>b</sup>.

<sup>a</sup> IFAPA Las Torres Tomejil, Área de Protección de Cultivos, Carretera Sevilla-Cazalla Km 12.2, Alcalá del Río, 41200 Seville, Spain.

<sup>b</sup> MycSA, UR1264, INRA, 71 Avenue Edouard Bourleaux, F-33883 Villenave d'Ornon Cedex, France.

\* corresponding author: ana.aguado@juntadeandalucia.es

### **INTRODUCTION**

Maize is one of the most important agricultural commodities in the world, and it is used as the major ingredient of dietary staple for humans and animals. In North America and Europe, many maize products are consumed, including breakfast cereals, snacks, softdrinks and beer. Maize is also used to make animal feed. Fumonisin are considered to be hazardous to human and animal health<sup>1-4</sup>. *Fusarium verticillioides* (synonym, *Gibberella fujikuroi*) is one of the most important fungal pathogens in maize causing both pre- and post-harvest losses and being also capable of producing mycotoxins such as Fumonisin B<sub>1</sub> (FB<sub>1</sub>) B<sub>2</sub> (FB<sub>2</sub>) and B<sub>3</sub> (FB<sub>3</sub>), being frequently detected in healthy maize grains<sup>5,6</sup>. *F. verticillioides* can infect maize via several routes. The exposed silks are the main pathway for the fungus to naturally get into the ear and reach the kernels<sup>7</sup>. Insects or other biotic or abiotic agents creating kernels wounds also favour kernel infection<sup>8</sup>. Systemic infection through the seed is another pathway. It starts from fungal conidia or mycelia that either are carried inside the seeds or on the seed surface, and the fungus develops inside the young plant, moving from the roots to the stalk and finally to the cob and kernels<sup>9</sup>. Systemic infection may also result from inoculum that survives in crop

1 residues in the soil <sup>10</sup> and penetrates young seedlings through the lateral roots and the  
2 mesocotyl <sup>9</sup>.

3  
4  
5 The prohibition of most of the chemical fungicides by the EU (Directive 91/414/CEE), due  
6 to adverse effects of their use on the environment and human health, their toxicity or  
7 because pathogens have become resistant to their active substances, have promoted  
8 research in the field of plant resistance inducers as an effective and sustainable alternative  
9  
10  
11  
12  
13  
14  
15 <sup>11</sup>.

16 Protection of maize seeds from infection of *F. verticillioides* by inoculation of antagonists  
17 has been experimented. It may reduce the rotting symptoms and fumonisin accumulation.  
18 Treatment of seeds with *Trichoderma harzianum* reduced the levels of fumonisins in  
19 different maize cultivars by 56-86% <sup>6</sup>. Fumonisin B<sub>1</sub> reduction reached up to 94% in grains  
20 treated with *Bacillus amyloliquefaciens*, and up to 81% in grains treated with  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

34 Breeding for resistance to *Fusarium* is the most economic and environmentally safe  
35 strategy. Genome-wide association study (GWAS) is a strategy successfully used to detect  
36 and identify quantitative trait loci (QTLs) and candidate genes involved in *F. verticillioides*  
37 resistance and low mycotoxin contamination synthesized by this fungus in maize. This tool  
38 allows to reveal novel sources of resistance to select maize lines in breeding programs  
39 quicker and more efficiently than the classic breeding. Recently, other researchers  
40 have got identified 8 QTLs and 43 genes associated with *F. verticillioides* seed rot (FSR)  
41 resistance through linkage mapping and GWAS, respectively <sup>14</sup>. By combining  
42 transcriptomic data with QTL, 24 candidate genes for resistance to *F. verticillioides* were  
43 identified, allowing to make possible the selection of genotypes with both low disease

1 severity and low fumonisin contamination <sup>15</sup>.  
2

3 The physiological health of the plantlets may also affect the development of the symptoms  
4  
5 <sup>9</sup> and induction of the priming state in the plant through the application of natural  
6 fertilizers on seeds could be another way to prepare plants against pathogen attacks.  
7  
8

9  
10 The aim of the present work was to characterize the influence of a commercial natural  
11 fertilizer based on vegetal extracts obtained from plant decoctions, Chamae on early  
12 colonisation of germinating seeds and plantlets by *F. verticillioides* and the  
13  
14 development of symptoms, including the fumonisin accumulation, and the influence of  
15  
16  
17  
18  
19 treatments on yield.  
20  
21  
22  
23  
24  
25

## 26 EXPERIMENTALS

### 27 28 29 30 **Seed treatments and inoculation**

31 The natural fertilizer Chamae (Saionaimer S.L.) used for seed treatments contains  
32 principal macronutrients (nitrogen, phosphorus, potassium), secondary macronutrients  
33 (calcium, magnesium, potassium, sodium and sulphur), and micronutrients (boron, cobalt,  
34 copper, iron, manganese, molybdenum and zinc). This natural product comes from the  
35 hydrolysis of crop residues through its decoction that favors the release of nutrients easily  
36 used by plants.  
37  
38  
39  
40  
41  
42  
43

44 Different dilutions of Chamae were applied on seeds of two maize varieties (MAS 68 K and  
45 LG-30681). MAS 68K from Maïsadour (Haut-Mauco, France) is a very late variety of dent  
46 maize for producing grains. LG-30681, from Limagrain (Saint Beuzire, France) is a very  
47 late variety suitable for both grains and forage. Both varieties were obtained in France.  
48  
49  
50

51 According to the breeder, LG-30681 is characterised by its high tolerance to *Fusarium*  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 ear rot. The seeds were surface sterilized. They were covered with 70% ethanol for 3 min, rinsed  
7  
8 with sterile milliQ water and submerged for 15 min in 2.5% sodium hypochlorite solution containing  
9  
10 40  $\mu\text{L}$  of Tween-20 100  $\text{mL}^{-1}$ . Hypochlorite was removed and the seeds were immersed again in  
11  
12 70% ethanol for 3 min, rinsed with sterile milliQ water 5 times and dried under a flux of sterilized air.  
13  
14 Surface-disinfected seeds were soaked in different dilutions of the natural fertilizer (1%, 0,01%,  
15  
16 0,005% v/v in demineralized water) for 20 hours at room temperature. For drying, they were spread  
17  
18 on sterilized filter paper and placed in a laminar flow hood for two hours. In controls, the treatments  
19  
20 were not applied to the surface disinfected seeds. Subsequently the seeds were plated on sterile  
21  
22 filter paper discs moistened with 1 mL of sterile demineralized water in 90 mm diameter Petri plates  
23  
24 at 25°C, without light, until germination.  
25  
26

27  
28 Germination of 40 seeds in each replicate and three replicates was evaluated for each dilution of  
29  
30 treatment with the natural product and control treatment in the two maize varieties. The percentage  
31  
32 of germinated seeds was estimated 20 and 48 hours after being deposited in Petri plate with  
33  
34 moistened paper.  
35

36  
37 The strain 0001 of *F. verticillioides* had been first isolated from symptomatic maize plants in Spain  
38  
39 and conserved as single spore isolate at the IFAPA Las Torres-Tomejil collection (Sevilla, Spain). It  
40  
41 was grown up in PDA plates at 25°C in the dark for 10 days. A suspension was prepared by  
42  
43 blending infested PDA with 5 mL of sterile distilled water in each plate. The spore suspension was  
44  
45 filtered through 4 layers of sterile cheesecloth. Spore concentration was determined using a  
46  
47 haemocytometer, and the suspension was diluted with sterile milliQ water to obtain a final  
48  
49 concentration of  $2 \times 10^6$  conidia  $\text{mL}^{-1}$ . When all the seeds had germinated, they were inoculated  
50  
51 pipetting 5  $\mu\text{L}$  of the spore suspension ( $2 \times 10^6$  conidia  $\text{mL}^{-1}$ ), in the area of the natural wound that  
52  
53 produces the exit  
54  
55  
56  
57  
58  
59  
60

1 of the rootlet during germination. 8 days after inoculation (d.a.i.), 20 seedlings repetition<sup>-1</sup>  
2  
3 of each treatment and variety were frozen, lyophilized and stored before quantification of  
4  
5 fumonisins and DNA isolation from *F. verticillioides*.  
6  
7  
8  
9  
10  
11

### 12 **Extraction and quantification of fumonisins from germinated and inoculated seeds**

13  
14 On the eighth day of inoculation, 4 g of freeze-dried ground germinated and inoculated  
15 seeds were extracted with 20 mL of methanol-water (3:1 v/v). After 15 min in agitation in  
16 an orbital shaker at 0.413 xg and centrifugation (3 min, 1013 xg), the supernatant was  
17 filtered through Whatman 2V filter paper. The filtrate was adjusted to pH 6.5 by adding  
18 NaOH 10N. Fumonisins were purified by using Bond Elut strong anion Exchange (SAX)  
19 Cartridges (Agilent), which were conditioned with 5 mL methanol and 5 mL  
20 Methanol/Water (3/1). Then 5 mL of sample was added to the cartridges. The samples  
21 were washed by passing through the column of 5 mL of methanol/water (3/1) and 3 mL of  
22 pure methanol. To elute the sample from the column, 10 mL of methanol with acetic acid  
23 (1%) were added. The samples were evaporated to dryness under a nitrogen stream and  
24 the pellets were dissolved in 200 µL of methanol before high performance liquid  
25 chromatography analysis with fluorescence detection. Just before injection (20µL),  
26 samples were derivatized using o-phthaldialdehyde (OPA) and β-mercaptoethanol mixture  
27 (40 mg of OPA are dissolved in 1 mL of methanol, 50 µL of β -mercaptoethanol and 5 mL  
28 of Borax 0.1M in water). Chromatographic separation was done with a C18 Kinetex  
29 column (150\*4.6 mm, 3.5µm) (Phenomenex, France) linked to an HPLC 1100 system  
30 (Agilent,) operating in isocratic mode. Mobile phase was composed of Methanol/Water,  
31 0.1M NaH<sub>2</sub>PO<sub>4</sub> (77/23, v/v) and pH was adjusted to 3.3 with orthophosphoric acid. Flow  
32 rate was 0.6 mL min<sup>-1</sup>. Excitation wavelength was 335 nm, and emission 440 nm. Total run  
33 time was 18 min.  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1 Quantification was performed by using external calibration with FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> standard  
2 solutions prepared from commercial pure powders (Romer Labs, Tulln, Austria).  
3  
4  
5  
6

### 7 **Detection and quantification of total DNA from *F. verticillioides* in maize samples**

8 Twenty seedlings in each repetition, treatment and variety were collected eight days after  
9 inoculation. These samples were lyophilised and crushed by liquid nitrogen. 0.1 g of these  
10 tissues was used to DNA extraction by DNeasy® Plant Mini Kit according to the  
11 manufacturer instructions (Qiagen, Courtaboeuf, France).  
12  
13  
14  
15  
16  
17

18 The other seedlings not used to extract DNA were planted in pot as it is described in the part  
19 “Early stage of maize growth (biomass aerial weight and plants height) and died plants”. So,  
20 a second DNA extraction was carried on lyophilized stems and leaves of young plants from  
21 pots thirty-six days after inoculation to detect the fungus and to know the possibility of  
22 colonization of aerial part of plants. They were ground under liquid nitrogen in a mortar and  
23 total DNA were extracted according to the N-cethyl- NNN-trimethyl ammonium bromide  
24 (CTAB) procedure, as described in Barroso *et al.* <sup>16</sup> The concentration of nucleic acids (DNA)  
25 was determined using a ND-1000 NanoDrop spectrophotometer (NanoDrop Products,  
26 Wilmington, DE) .  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37

38 For each analyzed sample, DNA was amplified with primers designed to track *F. verticillioides*  
39 DNA. The pair primer Fum 1-654 ( 5'- CGGTTGTTTCATCATCTCTGA-3') and Fum1-1158 (5'-  
40 GCTCCCGATGTAGAGCTTGTT-3') was designed to amplify a 504-bp fragment (T<sub>m</sub>, 60°C)  
41 from the polyketide synthase gene FUM1 (GenBank accession number AF155773) of  
42 *F. verticillioides*.  
43  
44  
45  
46  
47  
48

49 To normalize the quantification as ng of DNA fungus ng<sup>-1</sup> of DNA maize, we also  
50 performed the quantification of gene maize actine (accession number 01238) MA-F  
51 (TCCTGACACTGAAGTACCCGATTG)/MA-R CGTTGTAGAAGGTGTGATGCCAGTT).  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 The corresponding melting temperature was 60°C, and the amplified fragment length was  
2  
3 95 base pairs (bp). To know the absolute amount of DNA of both, standard curves of cycle  
4  
5 thresholds were generated using known quantities of DNA pure of *F. verticillioides* or of  
6  
7 maize (from 0.002 to 20 ng).  
8

9  
10 Quantitative PCR experiments (qPCR) were performed in a LightCycler® 1.5 System  
11  
12 (Roche Applied Science, Meylan, France) and the QuantiFast SYBR Green PCR Kit  
13  
14 (Qiagen, Courtaboeuf, France). Each reaction mixture contained 20 ng of DNA (1 µL),  
15  
16 QuantiFast SYBR Green PCR Master Mix 1X, 5 mM MgCl<sub>2</sub>, and 0.5 mM of each primer.  
17

18 The amplification conditions consisted of a first denaturation step for 5 min at 95 °C,  
19  
20 followed by 40 cycles of 10 s denaturation at 95 °C, 30 s combined annealing/extension at  
21  
22 60 °C. The melting curve was generated using the following profile: 15 s at 95°C, 15 s at  
23  
24 65°C, and 15 s at 95°C with a 0.1 °Cs<sup>-1</sup> transition. Assays for each sample were performed  
25  
26 in triplicate.  
27

28  
29 Lack of non-specific qPCR amplification or primer-dimer formation was checked by melting  
30  
31 curve analysis in each run.  
32

### 33 34 **Early stage of maize growth (biomass aerial weight and plants height) and died** 35 36 **plants**

37  
38 To evaluate the evolution of the fungus from the seed to the aerial part of the plants and  
39  
40 consequences on the plant growth, 9 germinated seeds of each treatment were planted in  
41  
42 different pots (14x14x20 cm) filled with 2.5 L of a potting substrate based on blend of  
43  
44 weakly decomposed white sphagnum peat and clay granules (Substrate 5, Klasmann-  
45  
46 Deilman GmbH, Germany). The plants were cultivated for 28 days in a greenhouse.  
47  
48 Temperature and air humidity were recorded. Mean temperature during the cultivation  
49  
50 period was 22°C with maximum at 30.2°C and minimum at 15.0°C. Mean air humidity was  
51  
52 59.1 % with 84.5 % as maximum and 37.0 % as minimum. The substrate was regularly  
53  
54  
55  
56  
57  
58  
59  
60

1 humidified when it was necessary. Damping-off symptoms as died plants were followed all  
2 along the culture. Height (cm) and aerial biomass weight (g) of plants were measured at  
3  
4 along the culture. Height (cm) and aerial biomass weight (g) of plants were measured at  
5  
6 the end of this growing period.  
7  
8  
9

### 10 **Number of productive plants, plant height and yield**

11  
12 An assay under field conditions was performed in Alcal del Rio (Seville, Spain). The soil  
13 had the following characteristics: texture silty clay, with a percentage of clay 46.4%, silt  
14 46.7% and sand 8.9%. The percentages of N total Kjeldahl, C.O. and M.O. are 0,12%,  
15 2,3% and 0,78% respectively, with a pH of 8,28 and the conductivity was 1,5 mS/cm.  
16  
17 Cation exchange capacity was (meq/100 g) 23.5% with Ca at 8.86%, Mg at 11.06%, K at  
18 1.26%, Na at 2.02. The percentage of base saturation is 99%, exchangeable sodium 6%,  
19 carbonates 20.6%, active limestone 11.6%, and the wealth in P is 21.7 ppm and in K 419  
20 ppm.  
21  
22  
23  
24  
25  
26  
27  
28  
29

30 The normal agronomic techniques were adopted. Briefly, the previous crop was maize,  
31 and the field was ploughed each year.  
32  
33

34 The sowing was performed on March 17th, 2017, using pneumatic testing machines at a  
35 high density and subsequent removal of plants required to obtain a density of 95,000-  
36 100,000 plants ha<sup>-1</sup>.  
37  
38  
39

40 The different treatments were: treated seed with dilutions of natural fertilizer Chamae to  
41 0.01% by imbibitions during 20 hours one day before sowing and seed without any  
42 treatment of two different varieties (MAS 68 K and LG-30681). The design was a random  
43 blocks with four replicates. Each repetition consisted on two rows 0.75 cm apart and 7 m  
44 of length.  
45  
46  
47  
48  
49  
50

51 When the plant reached its male flowering, at which time it has already its definitive size,  
52 the number of productive plants was measured by counting plants with standard height  
53 and regular ear size in each  
54  
55  
56  
57  
58  
59  
60

1 replicate in one row. The height of ten plants was also measured.

2  
3 The harvest was performed on August 28th, 2017, using harvest testing machines and the  
4  
5 yield was quantified in kg ha<sup>-1</sup>.  
6  
7  
8  
9

### 10 **Statistical analysis**

11 An ANOVA by variety for treatments was performed for all measured variables (Table 1  
12 and 4). In case of interaction between variety and treatment was not significant, mean  
13  
14 values of characters were average of two maize varieties. When interaction were  
15  
16 significant, the variables were analysed by ANOVA in each variety. Means were compared  
17  
18 using the Least Significant Difference test (LSD) at  $P \leq 0.05$  and  $P \leq 0.01$ . Percentage data  
19  
20 were transformed ( $\arcsin \sqrt{Y/100}$ ) and ANOVA was used to test for statistically significant  
21  
22 effects of independent variables. All statistical analyses were performed with Statistix 9.0  
23  
24 (Analytical Software, Tallahassee, FL).  
25  
26  
27  
28  
29  
30  
31

## 32 **RESULTS**

33 The study was conducted to evaluate the influence of a natural fertilizer applied on seed in  
34 order to control fumonisin production, *F. verticillioides* development and agronomic  
35 parameters as germination, plants height, died plants, aerial biomass weight of plants and  
36 yield. The result showed that fumonisin production and *F. verticillioides* development were  
37 reduced on inoculated maize plants by the application of natural fertilizer on seeds and the  
38 impact of this fertilizer on agronomic parameters; however the highest concentration of this  
39 natural fertilizer had a negative impact on the number of died plants, but lower  
40 concentrations minimized this negative impact.  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52

### 53 **Seed germination**

54  
55  
56  
57  
58  
59  
60

1 Treatments effect on seed germination is reported in Table 1 and Table 2. There was a  
2 significant interaction between variety and treatment and the effect due to both, variety and  
3 treatment (Table 1).  
4  
5  
6  
7

8 The data indicated that 20 hours after placing the seeds on watered filter paper in Petri  
9 plates, no germination was observed in controls (non treated seed) of both maize varieties  
10 whereas the germination rates in treated seeds were between 80 and 90% for the maize  
11 variety MAS 68 K and between 50 and 60% for LG-30681 (Table 2). The differences  
12 between the two varieties were significant for all the doses of treatment with the natural  
13 fertilizer used for priming (Table 1 and Table 2.). After 48 hours, between 95 and 100% of  
14 the seeds germinated (Data not shown). The treatment did not affect the germination  
15 power of seeds and no difference between the varieties was significant, but it was  
16 observed that seed treatments anticipated the germination.  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29

### 30 ***F. verticillioides* growth and fumonisin production in inoculated seedlings**

31 The fumonisins B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub> and *F. verticillioides* DNA concentration are shown in Table  
32 1, Fig. 1. and Fig. 2. The variation factors maize variety, treatments and the interaction  
33 between both were significant to FB<sub>1</sub>, FB<sub>2</sub> and fungus DNA quantity (Table 1). In the case of  
34 FB<sub>3</sub> the only significant factor was treatment (Table 1).  
35  
36  
37  
38  
39

40 8 days after *F. verticillioides* inoculation, fumonisin concentrations were significantly higher  
41 ( $P \leq 0.05$ ) on seedling from MAS 68 K variety than in LG 30681 variety (Fig. 1). In MAS 68  
42 K, all dilutions of the natural product reduced significantly ( $P \leq 0.05$ ) fumonisin accumulation  
43 (FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>) with regard to inoculated control (Fig. 1.). In the case of LG 30681  
44 variety, the accumulation of these mycotoxins showed lower values than in the other  
45 variety and it was not affected by treatments on seeds (Fig. 1.). Regarding the  
46 proportion of different fumonisins detected, FB<sub>1</sub> had the highest concentrations, followed  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 by FB<sub>2</sub> and finally FB<sub>3</sub> (Fig. 1.).

2  
3  
4 The same 20 seedlings repetition<sup>-1</sup> were used to quantify fungus DNA (Fum 1 gene) as an  
5  
6 estimation of the *F. verticillioides* biomass. Significant differences ( $P\leq 0.05$ ) for this  
7  
8 parameter were explained by effects of seed treatments and maize varieties (Table 1 and  
9  
10 Fig. 2). An interaction between variety and treatment was observed (Table 1). Significant  
11  
12 higher quantity of *F. verticillioides* DNA was observed in MAS 68 K seedling than in the  
13  
14 variety LG 30681 (Fig. 2.). Significant lower amount ( $P\leq 0.05$ ) of fungal DNA was observed  
15  
16 in MAS 68 K variety treated with 0.01% of natural fertilizer (Fig. 2.). In LG 30681 seedlings  
17  
18 variety, the 3 doses reduced significantly ( $P\leq 0.05$ ) the amount of fungal DNA measured  
19  
20 (Fig. 2.). We note that in negative control seedlings, fungal DNA was not detected. It  
21  
22 indicates that there had not been cross contamination during inoculation or incubation  
23  
24 (Data not shown).  
25  
26  
27  
28  
29

### 30 **Plant height, dead plants percentage and weight of aerial biomass of inoculated** 31 **plants with *F. verticillioides***

32  
33  
34 No interaction between variety and treatment was detected (Table 1), so the results for  
35  
36 these parameters are represented as the means of the two varieties (Table 3).  
37

38  
39 One month after the transplantation of the seedlings, the seed treatments and inoculation  
40  
41 with *F. verticillioides* had not a significant effect on the plant height and weight of aerial  
42  
43 biomass of plants under control condition (Table 1 and Table 3). Nevertheless, we  
44  
45 observed that the dilutions 1% and 0,05% of seed treatments incremented significantly  
46  
47 ( $P\leq 0.05$ ) the percentage of dead plants (Table 1 and Table 3). In both inoculated and non-  
48  
49 inoculated controls, dead plants were not observed (Table 3). The dilution 0.01% of seed  
50  
51 treatment showed dead plants but this parameter was not significantly different from the  
52  
53 inoculated and non-inoculated control (Table 1 and Table 3).  
54  
55  
56  
57  
58  
59  
60

1 No significant differences were observed among treatments for the weight of aerial biomass  
2  
3  
4 (Table 1 and Table 3).  
5  
6  
7

### 8 **Number of productive plants, plant height and yield**

9

10 To determine the effect on seeds of natural fertilizer in a field experiment, we chose the  
11 0.01% dilution applied on seed because it showed the best behaviour in the evaluated  
12 parameters under controlled conditions (germination, fumonisin accumulation, plant death  
13 and biomass weight aerial), compared with the effect of control seed on number of  
14 productive plants, plant height and yield in the two maize varieties (MAS 68 K and LG  
15 30681) under field conditions (Table 4 Table 5).  
16  
17  
18  
19  
20  
21  
22

23 Eighty-four days after sowing, the number of productive plants coming from treated seeds  
24 was significantly lower ( $P \leq 0.05$ ) than in non treated controls (Table 4 and Table 5).  
25  
26

27 Three months after sowing, when the plants were flowering, the plant height was not  
28 significantly different among different treatments (Table 4 and Table 5).  
29  
30

31 The yield, estimated as  $\text{kg ha}^{-1}$  at the end of the season, was not significantly affected by  
32 the seed treatment (Table 4 and Table 5).  
33  
34  
35  
36  
37

## 38 **DISCUSSION**

39

40 Maize fumonisins contamination poses a serious risk to human and animal health. The  
41 study of new techniques for its control, overall if they are respectful with the environment  
42 and they are low cost, is a priority for the sustainable control of this serious problem. The  
43 aim of this work has been the study of the influence of the priming state on maize plants,  
44 caused by the application of Chamae in the seed state.  
45  
46  
47  
48  
49  
50

51 Seed priming is a pre-sowing technique for regulating the germination process by  
52 triggering the pre-germinative metabolism that is normally activated during the early phase  
53  
54  
55  
56  
57  
58  
59  
60

1 of germination. It is proposed as a way to achieve faster and better seed germination.

2  
3 Among the various priming techniques, biopriming involves a soaking treatment with  
4  
5 beneficial microorganisms or bioactive molecules such as phytohormones <sup>17</sup>. In most cases,  
6  
7 previous researches showed a greater rate of germination in treated seed <sup>18</sup>. However, the  
8  
9 results obtained by various priming techniques in seeds are heterogeneous according to the  
10  
11 different species, the quality of the seed or the conditions of germination, among other  
12  
13 parameters <sup>19</sup>.

14  
15  
16 Molecular techniques which are used for the improvement of *F. verticillioides* resistance and  
17  
18 fumonisins production are efficient in breeding programs. They allow quick selection of lines  
19  
20 in maize. However, the complex genetic basis of these characters and the great  
21  
22 environment effect in their phenotypic expression, make the location of QTLs and the  
23  
24 efficiency of marker-assisted selection (MAS) more difficult <sup>20</sup>.

25  
26  
27 In this sense, the seeds priming technique could improve or help the effect obtained  
28  
29 through breeding of resistant maize varieties, being interesting for studies on an  
30  
31 intergrated disease management combining both techniques.

32  
33  
34 In the present study, we used three different dilutions of Chamae, soaking of maize seeds  
35  
36 for 20 hours as priming treatment. Due to the very high percentages of germination rates of  
37  
38 controls, no improvement of this trait was observed. However, all the treatments decreased  
39  
40 the germination delay by at least one day, in comparison with the control plants. In addition  
41  
42 to the beneficial effect on germination, priming is also proposed as a way to produce  
43  
44 tolerant plants against various stresses <sup>21</sup> by the initiation in the plant of a "preparation"  
45  
46 state that does not confer resistance in itself but allows the acceleration of induction of the  
47  
48 defences, once the plant faces such stress. The plant is then able to respond better than  
49  
50 other plants that have not experienced it <sup>22</sup>. Thus, an advantage of this technique is that it  
51  
52 does not entail the costs normally associated with the activation of an inducible  
53  
54  
55  
56  
57  
58  
59  
60



1 defence response, since the defensive response is triggered only if the plant recognizes  
2 some of these types of stress, and without major ecological or physiological effects <sup>23</sup>.  
3  
4 Systemic infection of maize by *F. verticillioides* could be through the seeds via fungal  
5 conidia or mycelia that are carried inside the seeds or coming from inoculums that survives  
6 in crop residues in the soil. This is an infection route of ears that have been proposed by  
7 several authors <sup>9,10</sup>. *F. verticillioides* is also considered as a “root pathogen” responsible of  
8 damping-off symptoms <sup>24</sup>. To determine whether plants might respond to priming agents at  
9 the seed stage, in the present study, we infected seedlings of two maize varieties with  
10 spores of a Spanish strain of *F. verticillioides*. We observed that the amount of DNA from  
11 the fungus was significantly lowered with the different dilutions of Chamae applied on  
12 seeds, with a different behaviour of the two maize varieties used. The amount of fungus  
13 DNA was three-fold lower in the variety LG-30681; it is known to be more tolerant than  
14 variety MAS 68 K. This lower colonization of *F. verticillioides* in a tolerant variety than in a  
15 susceptible variety agree with the observation of Wu *et al.* <sup>24</sup>. The three-fold lower  
16 development of *F. verticillioides* led to a more than ten-fold lower fumonisin  
17 accumulation. These micotoxins are known to contribute to virulence in seedlings grown  
18 from inoculated maize seeds <sup>25,26</sup>. The partial resistance to colonization at seedling and  
19 limitation of fumonisin production might be a component of the claimed low susceptibility of  
20 LG-30681 to Fusarium ear rot. The same results were observed also in adult plants and  
21 not only at the seedling stage <sup>27</sup>.

22  
23 Previous studies showed high level of variability between susceptible and resistant maize  
24 lines for the respond to *F. verticillioides* infection. Although both presented similar  
25 functional categories of respond genes to infection. Resistant lines had basic defence to  
26 the fungus because genes were highly transcribed before infection. However, in  
27 susceptible maize lines, the genes were induced after infection. These results suggest that  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 plant basal genes in seed tissue could decrease colonization and fumonisin synthesis by the  
2 fungus<sup>27, 28</sup>.

3  
4  
5 The dilution of Chamae to 0.01% applied on seeds of the susceptible variety (MAS 68 K) was  
6 able to reduce significantly the level of fumonisin accumulation and the biomass of *F.*

7  
8  
9  
10 *verticillium* in seedlings during the first days following the inoculation. The level of  
11 contamination of seedlings was reduced to the same level as that of the tolerant untreated  
12 variety. The tolerant variety did not show any significant response to the treatment for the  
13 fumonisin accumulation, but the quantity of fungal biomass was significantly lowered in  
14 seedlings treated with the different dilutions of Chamae. These results suggest that the  
15 treatments might favour defence responses in seedlings when they are inoculated with the  
16 fungus *F. verticillioides*. Interestingly, plant response did not induce a reaction of the used  
17 strain of *F. verticillioides* through higher production of fumonisins, but a lower rate of fumonisin  
18 accumulation per unit of fungal biomass. Production of oxidative stress protectors is known to  
19 be activated (accumulation of stress-related proteins, constitutive expression of  
20 pathogenesis-related proteins and antioxidant enzyme activities) as maize responses to *F.*  
21 *verticillioides* infection in embryos and adult plants, triggering maize resistance towards this  
22 fungus<sup>29,30</sup>. Ferrigo *et al.*<sup>31</sup> observed divergent behaviours in *F. verticillioides* populations,  
23 face to oxidative stress induced by H<sub>2</sub>O<sub>2</sub>. Other strains could react differently than the  
24 Spanish- one tested here, and a large panel of strains should be tested in further works.

25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
Mycotoxin contamination of feed often begins in the field but this could continue at harvest,  
transportation and storage, depending on environmental conditions. Thus, the most effective  
way to prevent mycotoxin occurrence is to limit the biosynthesis by the fungus when the crop  
is cultivated<sup>13</sup>.

1 After infection, *F. verticillioides* might develop systemic colonization of young plants. In the  
2  
3 present work, after planting of seedling in a horticulture substrate in pots, we obtained  
4  
5 either plants that developed normally without symptoms or plants that died before they  
6  
7 reached their first month of growth. Because of the possibility of plant colonization by the  
8  
9 fungus, we wanted to know if after inoculation in seedlings, this colonization of the stem  
10  
11 had occurred when the plants were already 1 month old. Therefore, the extraction of DNA  
12  
13 from the stem of the plants was carried out and the presence of *F. verticillioides*' DNA was  
14  
15 studied by quantitative-PCR. Whereas no symptoms were observed on plants whatever  
16  
17 the treatments and fungus, DNA was not detected by PCR in the stems of the inoculated  
18  
19 plants after surface sterilisation (Data not showed). The aerial biomass was not significantly  
20  
21 affected in the plants from treated seeds inoculated with *F. verticillioides*. According to the  
22  
23 work of Howard <sup>32</sup> and Duncan and Howard <sup>33</sup>, *F. verticillioides* does not form penetrating  
24  
25 structures that break the epidermis, which can hinder a systemic infection. Oren *et al.* <sup>9</sup>  
26  
27 observed asymptomatic systemic infection characterized by infection of only certain  
28  
29 tissues, intercellular growth of a limited number of fungal hyphae, and reproduction of the  
30  
31 fungus in a few cells without invasion of other cells. In the stems, they detected only few  
32  
33 mycelium of *F. verticillioides* green fluorescent protein expressing transgenic isolate. We  
34  
35 cannot exclude that such asymptomatic colonization occurred in the present experiment  
36  
37 with levels of mycelium that were not detectable. In our experiment, necrotrophic symptoms  
38  
39 were not observed in non-treated control whereas they were shown at the lowest dilutions  
40  
41 or highest concentration of Chamae. The fungus probably used the nutrients from the  
42  
43 fertilizer coating the seeds for its external development and then rotted mesocotyl and main  
44  
45 root cells with massive production of fungal mycelium as was observed by Oren *et al.* <sup>9</sup> in  
46  
47 physiologically weakened plants. In their study, this always led to stop the development  
48  
49 of the plants and their death. This negative effect might be  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 modulated under other circumstances of *F. verticillioides* inoculum potential and soil  
2 environment, where the space could be occupied before by competitors.  
3

4  
5 The defences induced by seed treatments may be associated to a plant energy cost that  
6 could result into losses of productive plants, production or even plant size<sup>34</sup>. Benefits from  
7 induced resistance responses via the application of different products might be limited by  
8 the inherent costs of defence<sup>35</sup>. The costs associated with direct activation of defences  
9 also could be associated with loss of yield or detrimental effects on other agronomic  
10 factors of plants. Such costs were observed with direct activation of the defense system in  
11 plants<sup>21</sup>, but not when a priming state was induced through application jasmonic acid on  
12 seeds<sup>19</sup>. In the present work, the fertilizer applied on seed had a minimal impact on the  
13 height and the aerial biomass weight of 1 month old plants and it did not affected final yield  
14 (kg ha<sup>-1</sup>), despite a lowering of the number of plant with standard height and regular ear  
15 size (productive plants) in each repetition.  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

## 31 **CONCLUSION**

32  
33  
34  
35  
36 In conclusion, according to our results, provoking a priming state by applying the natural  
37 fertilizer Chamae on maize seeds could improve germination and limit the colonization of  
38 seedlings by *F. verticillioides* and its associated fumonisin accumulation. However,  
39 depending on the dilution of fertilizer applied, further plant death was observed, and finally  
40 the number of productive plants under field conditions was lowered, but without affecting  
41 the final yield, suggesting a higher level of biomass production per plant. This situation can  
42 be amended with an increase of planting density. The 0.01% dilution of the seed treatment  
43 is the most recommended since it improved the germination, reduced the amount of  
44 fungus DNA and fumonisins production by fungus, did not increment significantly  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 death plants and did not reduce the yield. Therefore, the priming can be recommended as  
2  
3 a low cost and effective technique in the control of the fumonisins production and the  
4  
5 development of *F. verticillioides* from seed infection, which does not affect the crop  
6  
7 production.  
8  
9

## 12 ACKNOWLEDGEMENTS

16 The authors would like to thank the technical staff of the Mycology and Food Safety Unit  
17  
18 (MycSA-INRA, Bordeaux, France) for their assistance.  
19  
20  
21  
22

## 23 References

- 24  
25 [1] Franceschi S, Bidoli E, Barón A and Vecchia CL, Maize and risk of cancers of the oral  
26  
27 cavity, pharynx, and esophagus in northeastern Italy. *J Natl Cancer Inst.* **82**: 1407–  
28  
29 1411 (1990).  
30  
31  
32 [2] Gelderblom WCA, Kriek N, Marasas WFO and Thiel PG, The effect of fumonisin B<sub>1</sub> on  
33  
34 the growth of bacteria. *Carcinogenesis* **12**: 1247–1251(1991).  
35  
36 [3] Norred WP and Voss KA, Toxicity and role of fumonisin in animal diseases and human  
37  
38 esophageal cancer. *J Food Protect* **57**: 522-527 (1994).  
39  
40 [4] Pitt JI and Tomaska L, Are mycotoxins a health hazard in Australia?. *Food Aust J* **53**:  
41  
42 545–559 (2001).  
43  
44 [5] Niderkorn V, Morgavi DP, Aboab B, Lemaire M and Boudra H, Cell wall component and  
45  
46 mycotoxin moieties involved in the binding of fumonisin B<sub>1</sub> and B<sub>2</sub> by lactic acid  
47  
48 bacteria. *J Appl Microbiol* **108**: 977-985 (2009).  
49  
50 [6] Nayaka SC, Niranjana SR, Shankar ACU, Raj SN, Reddy MS, Prakash HS and  
51  
52 Mortensen CN, Seed biopriming with novel strain of *Trichoderma harzianum* for the  
53  
54  
55  
56  
57  
58  
59  
60

- 1 control of toxigenic *Fusarium verticillioides* and fumonisins in maize. *Arch*  
2  
3  
4 *Phytopathology Plant Protect* **43**: 264–282 (2010).
- 5  
6 [7] Munkvold GP and Carlton WM, Influence of inoculation method on systemic *Fusarium*  
7  
8 *moniliforme* infection of maize plants grown from infected seeds. *Plant Dis* **81**: 211-216  
9  
10 (1997).
- 11  
12 [8] Munkvold GP, Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears.  
13  
14 *Eur J Plant Pathol* **109**: 705–713 (2003).
- 15  
16 [9] Oren L, Ezrati S, Cohen D and Sharon A, Early events in the *Fusarium verticillioides*–  
17  
18 maize interaction characterized by using a green fluorescent protein-expressing  
19  
20 transgenic isolate. *Appl Environ Microb* **69**: 1695–1701 (2003).
- 21  
22 [10] Rheeder JP and Marasas WFO, *Fusarium* species from plant debris associated with  
23  
24 soils from maize production areas in the Transkei Region of South Africa.  
25  
26 *Mycopathologia* **143**: 113-119 (1998).
- 27  
28 [11] van Aubel G, Buonatesta R, and Van Cutsem P, COS-OGA: A novel oligosaccharidic  
29  
30 elicitor that protects grapes and cucumbers against powdery mildew. *Crop Prot* **65**:  
31  
32 129-137 (2014).
- 33  
34 [12] Pereira P, Nesci A, Castillo C and Etcheverry M, Impact of bacterial biological control  
35  
36 agents on fumonisin B<sub>1</sub> content and *Fusarium verticillioides* infection of field-grown  
37  
38 maize. *Biol Control* **53**: 258-266 (2010).
- 39  
40 [13] Dalié D, Pinson-Gadais L, Atanasova-Penichon V, Barreau C, Deschamps A and  
41  
42 Richard-Forguet F, Impact of *Pediococcus pentosaceus* strain L006 and its metabolites  
43  
44 on fumonisin biosynthesis by *Fusarium verticillioides*. *Food Control* **23**: 405-411(2012).
- 45  
46 [14] Ju M, Zhou Z, Mu C, Zhang X, Gao J, Liang Y, Chen J, Wu Y, Li X, Wang S, Wen J,  
47  
48 Yang L, Jianyu Wu J, Dissecting the genetic architecture of *Fusarium verticillioides*  
49  
50 seed rot resistance in maize by combining QTL mapping and genome-wide association  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1 analysis. *Sci Rep* **7**: 1-11 DOI: 10.1038/srep46446 (2017)
- 2
- 3
- 4 [15] Maschietto V, Colombi C, Pirona R, Pea G, Strozzi F, Marocco A, Rossini L, Lanubile
- 5 A, QTL mapping and candidate genes for resistance to Fusarium ear rot and fumonisin
- 6 contamination in maize. *BMC Plant Biology*: **17:20**: 1-21. DOI 10.1186/s12870-017-
- 7 0970-1 (2017).
- 8
- 9
- 10
- 11
- 12
- 13 [16] Barroso G, Perennes D and Labarère JA, 'miniprep' method for RFLP analysis and
- 14 dsRNAs detection perfected in the cultivated fungus *A. aegerita*. In: Science and
- 15 Cultivation of Edible Fungi, ED. by Elliot TJ. Rotterdam: Balkema, AA, pp. 87-94.
- 16 (1995).
- 17
- 18
- 19
- 20
- 21
- 22 [17] Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D and Balestrazzi A,
- 23 Seed priming: state of the art and new perspectives. *Plant Cell Report* **34**: 1281-1293
- 24 (2015).
- 25
- 26
- 27
- 28
- 29 [18] Di Girolamo G and Barbanti L, Treatment conditions and biochemical processes
- 30 influencing seed priming effectiveness. *It J Agron* **7**: 178-188 (2012).
- 31
- 32
- 33 [19] Worrall D, Geoff HH, Jason PM, Marcin G, Croft P, Taylor Jane ET, Nigel DP and
- 34 Roberts RM, Treating seeds with activators of plant defence generates long-lasting
- 35 priming of resistance to pests and pathogens. *New Phytol* **193**: 770-778 (2012).
- 36
- 37
- 38
- 39 [20] Robertson-Hoyt LA, Jines MP, Balint-Kurti P, Kleinschmidt CE, White DG, Payne GA,
- 40 Maragos CM, Molnar TL, Holland JB, QTL mapping for Fusarium ear rot and fumonisin
- 41 contamination resistance in two maize populations. *Crop Sci* **46**:1734–43 (2006)
- 42
- 43
- 44
- 45
- 46 [21] van Hulten M, Pelsler M, van Loon LC, Pieterse CMJ and Ton J, Costs and benefits of
- 47 priming for defense in Arabidopsis. *P Nat Acad Sci* **103**: 5602–5607 (2006).
- 48
- 49
- 50 [22] Conrath U, Beckers GJM, Flors V, García-Agustín P, Jakab G, Mauch F and
- 51 MauchMani B, Priming: getting ready for battle. *Mol Plant Microbe In* **19**: 1062–1071
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

- 1 (2006).  
2  
3  
4 [23] Frost CJ, Mescher MC, Dervinis C, Davis JM, Carlson JE and de Morales CM,  
5  
6 Priming defense genes and metabolites in hybrid poplar by the green leaf volatile cis-  
7  
8 3hexenyl acetate. *New Phytol* **180**: 722-734 (2008).  
9  
10 [24] Wu L, Wang X, Xu R and Li H, Difference between resistant and susceptible maize to  
11  
12 systematic colonization as revealed by DsRed-labeled *Fusarium verticillioides*. *The*  
13  
14 *Crop J* **1**: 61–69 (2013).  
15  
16 [25] Desjardins AE, Plattner RD, Nelsen TC and Leslie JF, Genetic analysis of fumonisin  
17  
18 production and virulence of *Gibberella fujikuroi* mating population A (*Fusarium*  
19  
20 *moniliforme*) on maize (*Zea mays*) seedlings. *Appl Environ Microb* **61**: 79-86 (1995).  
21  
22 [26] Glenn AE, Zitomer NC, Zimeri AM, Williams LD, Riley RT and Proctor RH,  
23  
24 Transformation-mediated complementation of a FUM gene cluster deletion in *Fusarium*  
25  
26 *verticillioides* restores both fumonisin production and pathogenicity on maize seedlings.  
27  
28 *Mol Plant Microbe In J* **21**: 87-97 (2008).  
29  
30 [27] Lanubile A, Logrieco A, Battilani P, Proctord RH, Marocco A, Transcriptional changes  
31  
32 in developing maize kernels in response to fumonisin-producing and nonproducing  
33  
34 strains of *Fusarium verticillioides*. *Plant Sci* **210**: 183– 192 (2013).  
35  
36 [28] Lanubile A, Bernardi J, Marocco A, Logrieco A, Paciolla C, Differential activation of  
37  
38 defense genes and enzymes in maize genotypes with contrasting levels of resistance  
39  
40 to *Fusarium verticillioides*, *Environ Exp Bot* **78**: 39–46 (2012).  
41  
42 [29] Maschietto V, Lanubile A, De Leonardis S, Marocco A, Paciolla C, Constitutive  
43  
44 expression of pathogenesis-related proteins andantioxydant enzyme activities triggers  
45  
46 maize resistance towards *Fusarium verticillioides*. *J Plant Physiol* **200**: 53-61 (2016).  
47  
48 [30] Campo S, Carrascar M, Coca M, Abian J and San Segundo B, The defense response  
49  
50 of germinating maize embryos against fungal infection: a proteomics approach.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1 *Proteomics* **4**: 383 – 396 (2004).

2  
3  
4 [31] Ferrigo D, Raiola A, Bogialli S, Bortolini C, Tapparo A and Causin R, In Vitro  
5  
6 Production of Fumonisins by *Fusarium verticillioides* under oxidative stress induced by  
7  
8 H<sub>2</sub>O<sub>2</sub>. *J Agr Food Chem* **63**: 4879-4885 (2015).

9  
10 [32] Howard RJ, Breaching the outer barriers—cuticle and cell wall penetration, In: Carroll  
11  
12 G and Tudzynski P editors. *Plant Relationships* (Vol. 5A): 43–60. Springer-Verlag, New  
13  
14 York (1997).

15  
16 [33] Duncan KE and Howard RJ, Biology of maize kernel infection by *Fusarium*  
17  
18 *verticillioides*. *Mol Plant Microbe In J* **23**: 6–16 (2010).

19  
20 [34] Cipollini D, Purrington CB and Bergelson J, Costs of induced responses in plants.  
21  
22  
23 *Basic Appl Ecol* **4**: 79-89 (2003).

24  
25 [35] Heil M, Hilpert A, Kaiser W and Linsenmair KE, Reduced growth and seed set  
26  
27 following chemical induction of pathogen defence: does systemic acquired resistance  
28  
29 (SAR) incur allocation costs? *J Ecol* **88**: 645-654 (2000).

1  
2  
3  
4  
5

6 Table 1. Analysis of Variance (ANOVA) for the studied parameters under controlled conditions: seed germination, fumonisins production (FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>), quantification  
7 of *F. verticillioides* DNA, height and weight of aerial parts of 1 month old plants

8 Parameters																	
9 Germination Rate				Fumonisin concentration						DNA quantifications		Percentage of died plants days after inoculation		Plant Height (cm)		Biomass weigh (gr)	
10 Source variation	df <sup>a</sup>	MS <sup>b</sup>	P	FB1	P	FB2	P	FB3	P	MS <sup>b</sup>	P	MS <sup>b</sup>	P	MS <sup>b</sup>	P	MS <sup>b</sup>	P
11 Treatments (T)	3	8454.51	0,0000***	12305.1	0.0121*	1583.96	0.0041**	40.55	0.0274*	0.00607	0.0041**	0.0038	0.0222*	68.18	0.3978	3.17	0.6908
12 Variety (V)	1	1926.04	0,0000***	73444.4	0.0001***	8866	0.0000***	226.97	0.4335	0.09401	0.0000***	0.0006	0.0000***	81.34	0.2724	3.05	0.4714
13 Repetitions (R)	2	0.26	0,9917	200.1	0.9184	211.66	0.4144	5.42	0.1055	0.00140	0.4144	0.0025	0.4236	91.62	0.2623	11.98	0.1506
14 T X V	3	232.29	0,0032**	9968.6	0.0245*	1452.97	0.0058**	37.20	0.6091	0.00122	0.0058**	0.0006	0.5181	48.03	0.5656	6.98	0.3321
Error	14	31.21		2334.7		225.47		5.77		0.00153		0.0009		63.21		5.61	

15 \* $P < 0.05$ , \*\*  $P < 0.001$ , \*\*\* $P < 0.0001$ ; <sup>a</sup> degree freedom; <sup>b</sup> Means square

16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47

Table 2. Effect of seed treatments on germination								
<i>Germination (%)</i>								
Varieties	LG-30681				MAS 68 K			
	Natural fertilizer dilutions (%)				Dilutions (%)			
Treatments	Control	1	0,01	0,005	Control	1	0,01	0,005
Natural fertilizer application	0 c	55.83 a	67.50 ab	65 a	0 c	83.33 ab	86.66 a	90 a
Different letters indicate significant differences according to LSD test ( $P \leq 0.05$ )								

Table 3. Effect of seed treatments on plant height weight of aerial biomass and died plants of maize inoculated with *Fusarium verticillioides* as average of two varieties (MAS 68 K and LG 30681)

Treatments	Control	Control inoculated	Natural fertilizer application dilutions (%) on seed and inoculated on seedling		
			1	0,01	0,005
	<i>Heigh plant (cm)</i>				
	65.00 a	61.00 a	59.86 a	59.83 a	55.67 a
	<i>Weigh of aerial biomass (g)</i>				
	9.13 a	8.69 a	7.65 a	7.69 a	7.44 a
	<i>Died plants</i>				
	0 a	0 a	49.60 c	11.10 ab	27.75 bc
Same letters indicate non-significant differences among treatments and different letters indicate significant differences according to LSD test ( $P \leq 0.05$ )					

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47

**Table 4. Analysis of Variance (ANOVA) for the studied parameters under field conditions: number of productive plants, height plants and yield**

Parameters								
Number of productive plants per block 84 days after sown								
Height plant (84 days after sown) in flowering								
Yield (Kg/Ha)								
Source variation	df <sup>a</sup>	MS <sup>b</sup>	P	MS	P	MS	P	
Treatments (T)	1	60.06	0.0355*	0.00045	0.7846	50457	0.8943	
Variety (V)	1	10.56	0.3272	0.00019	0.8594	7.2E+07	0.0002***	
Repetitions (R)	3	1.72	0.9102	0.00566	0.4387	7E+06	0.09	
T X V	1	3.06	0.5905	0.02038	0.091	3575722	0.2755	
Error	9	9.84		0.051		2738860		

\*P < 0.05, \*\*\*P < 0.0001; <sup>a</sup> degree freedom; <sup>b</sup> Means square

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47

Treatments	Treated seed with doses 0.01%	Non treated seed
	<i>Height plant (cm)</i>	
	2.71 a	2.71 a
	<i>Number of productive plants</i>	
	48.75 a	52.62 b
	<i>Yield (kg ha<sup>-1</sup>)</i>	
	10605 a	10505 a

Same letters indicate non-significant differences among treatments and different letters indicate significant differences according to LSD test ( $P \leq 0.05$ )

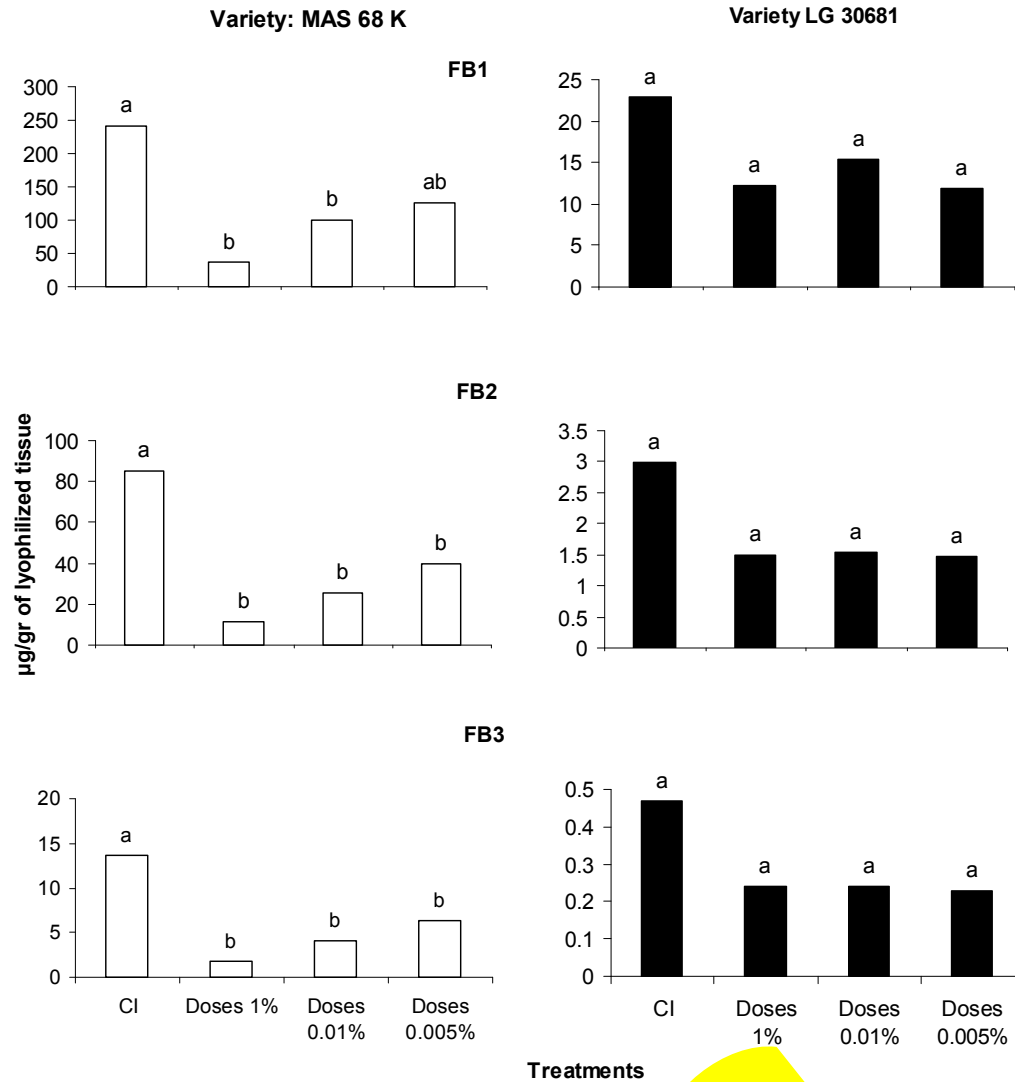


Figure 1. Accumulation of fumonisins: **FB<sub>1</sub>**, **FB<sub>2</sub>** and **FB<sub>3</sub>**, 8 days after inoculation by *Fusarium verticillioides* in seedlings of maize, previously treated with different doses of a natural fertilizer on seeds of 2 varieties (MAS 68 K and LG 30681). CI: control inoculated plants and non-treated seeds; Doses 1%: inoculated plants and seed treated with natural fertilizer diluted at 1% (v/v); Doses 0.01%: inoculated plants and seed treated with natural fertilizer diluted at 0.01% (v/v); Doses 0.005%: inoculated plants and seed treated with natural fertilizer diluted at 0.005% (v/v). Bars with the same letters indicate non-significant differences among treatments and bars with different letters indicate significant differences among treatments according to LSD test ( $P \leq 0.05$ ).

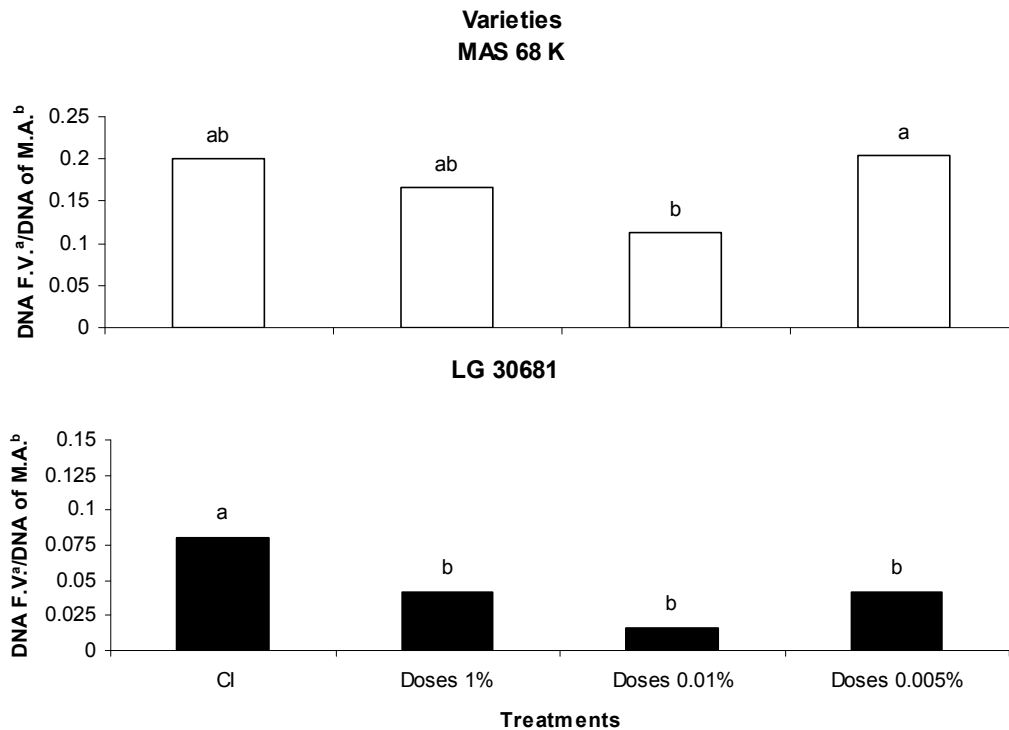


Figure 2. Quantification of *Fusarium verticillioides* DNA as the ratio *Fum1* gene amplification to *maize actin* gene amplification, in seedlings of two maize varieties (MAS 68 K and LG 30681), 8 days after inoculation.

CI: control inoculated plants and non-treated seeds; Doses 1%: inoculated plants and seed treated with natural fertilizer diluted at 1% (v/v); Doses 0.01%: inoculated plants and seed treated with natural fertilizer diluted at 0.01% (v/v); Doses 0.005%: inoculated plants and seed treated with natural fertilizer diluted at 0.005% (v/v). Bars with different letters indicate significant differences among treatments and varieties according to LSD test ( $P \leq 0.05$ ).