1 *Plant Pathology*

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6 **Occurrence of *Fusarium* species in maize kernels grown in Northwestern Spain**

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Abstract

Efforts are required to understand the epidemiology of the *Fusarium* disease by focusing more precisely on the relationship between environmental variables and the disease presence. The objectives of the present study were to monitor the occurrence of *Fusarium* species in maize kernels in Northwestern Spain in order to determine the potential risk of mycotoxin contamination, and to identify environmental traits affecting the composition of the *Fusarium* species identified.

The environmental mean of *F. verticillioides* presence ranged from 33 to 99 %, supporting the idea that the fumonisin contamination is the main maize-based feed and food safety concern in this area, although emerging mycotoxins such as moniliformin, fusaproliferin and beauvericin should be also taken into account. Under the particular environmental conditions of this region we must point out temperature and humidity in relation to the *Fusarium* spp. occurrence. We determine that warmer temperatures at later stages of kernel development and during kernel drying increase the frequency of *F. verticillioides* in maize kernels; while the presence of *F. subglutinans* is impacted by higher relative humidity at the silking stage and cooler temperatures during the kernel drying period. The management of sowing and harvest dates can be effective in order to modulate the fungal presence and growth.

Key words: *Fusarium*, *Zea mays*, fumonisin; environment; presence; kernel, silk

46 Molds belonging to the genus *Fusarium* are widely found infecting maize kernels in
47 temperate regions. The occurrence of *Fusarium* species is a food and feed safety
48 problem because most of them produce mycotoxins (Logrieco et al., 2003,). Symptoms
49 of mycotoxicosis depend on the type of mycotoxin, concentration, length of exposure
50 and characteristic of the exposed individual (e.g. age and health), but mycotoxins could
51 especially cause injuries in liver, kidneys, and immune, endocrine and/or nervous
52 systems (Bennett & Klich, 2003). They can be mutagenic and carcinogenic; potential
53 carcinogenic risk for some mycotoxins has been rated by the International Agency for
54 Research on Cancer (IARC, 1993). Therefore, legislation to limit the amount of some
55 mycotoxins has been implemented in many parts of the world (FAO, 2004) in order to
56 minimize human health risk.

57 Climatic conditions determine the predominance of a particular species or group
58 of species which cause different types of maize ear rot. In cooler temperate regions,
59 Gibberella ear rot is predominant and is mainly caused by *F. graminearum* and related
60 species such as *F. culmorum*, *F. cerealis* and *F. avenaceum* (Munkvold, 2003, Logrieco
61 et al., 2002, Bottalico, 1998). In warmer regions, Fusarium ear rot is prevalent and is the
62 result of kernel infection by *F. verticillioides* and other species of the *Gibberella*
63 *fujikuroi* complex, such as *F. proliferatum* and *F. subglutinans.* All these species are
64 mycotoxigenic and, depending on the particular species, can produce trichothecenes,
65 fumonisins and/or zearalenone, and other mycotoxin comparatively less important such
66 as moniliformin, beauvericin, fusaproliferin, fusaric acid or enniatins (Logrieco et al.,
67 2002, Jestoi, 2008). In Spain, maize kernel seemed to be predominantly infected by *F.*
68 *verticillioides* and in a lesser extent by *F. proliferatum*, both known as fumonisin
69 producers (Butron et al., 2006, Jurado et al., 2006, Arino et al., 2007). Significant
70 differences among years and locations for *Fusarium* spp. incidence in maize kernels has

71 been reported in many geographical areas (Bottalico, 1998, Goertz et al., 2010,
72 Boutigny et al., 2012, Covarelli et al., 2011). Bakan et al. (2002), analyzing kernel
73 infection by *Fusarium* ssp., found that *F. proliferatum* was more abundant in
74 northeastern Spain. Our experimental plots are located in northwestern Spain, where
75 climatic characteristics during kernel filling are very different from northeastern Spain
76 conditions, and those climatic differences could be responsible for differences in the
77 *Fusarium* species identified in the area (Marin et al. 1996; Butron et al., 2006).
78 Attending to the fumonisin contamination, Sanchis et al. (1995) had already pointed out
79 the potential fumonisin contamination in many Spanish corn-based products containing
80 both *Fusarium* species, while a previous papers from our group noted fumonisin
81 contamination of maize flours above the levels established in the European Regulation
82 (Butrón et al., 2006).
83 Although yearly and geographical variation in the diversity of *Fusarium* in
84 maize kernels has been noted, we have no information attending the environmental
85 traits affecting biodiversity other than the wetter regions seemed to favor greater
86 *Fusarium* contamination than the drier regions (Cantalejo et al. 1998). Therefore, the
87 objectives of the present study were: (i) to monitor the occurrence of *Fusarium* species
88 in maize kernels in Northwestern Spain in order to determine the potential risk of
89 contamination by several mycotoxins; and (ii) to identify environmental traits
90 associated with the variability in the *Fusarium* species composition in the area.

91 **Materials and methods**

92 **Field experiments.** Six maize hybrids derived from crosses among inbred lines EP39,
93 CM151, EP42 and EP47 were used to monitor the prevalence of *Fusarium* spp. on
94 maize kernels under natural infection. As corn borer attack has been associated to
95 increased kernel infection by fungus (Smith & White, 1988), two inbred lines (EP39
96 and CM151) were selected as resistant to the Mediterranean corn borer (*Sesamia*
97 *nonagrioides* Lef.) attack and the other two (EP42 and EP47) as susceptible (Santiago
98 et al., 2003). Hybrids were evaluated at early (end of April) and late (middle of May)
99 sowings in 2007 and 2008 in three locations in Northwestern Spain and were harvested
100 in two dates. Locations were Pontevedra (42º 24' N, 8º 38' W, 50 m above sea level)
101 and Barrantes (42º 30' N, 8º 46' W, 50 above sea level), both placed close to the coast,
102 and Valongo (42º 26' N, 8º 27' W, 500 above sea level), situated in the inlands.
103 Therefore, hybrids were evaluated in a total of 24 environments (combination of 2
104 years-3 locations-2 sowing dates-2 harvest dates). A split-plot design with three
105 replications was used for each trial (year-location-sowing combination); hybrids were
106 assigned to main plots and harvest times to sub-plots. Main plots consisted in two rows
107 with 13 two-kernel hills per row, rows being 0.80 m apart from each other and hills 0.21
108 m apart. After thinning the final density was around 60 000 plants ha^{-1}. Within each
109 plot, ears from one row (sub-plot) were harvested at the beginning of October (early
110 harvest) and from the other row one month later (late harvest). Harvested ears were
111 shelled and kernels were dried at 35 ºC for one week and maintained at 4 ºC and 50 %
112 humidity until analyses were performed.

113 **Environmental variables.** A meteorological station was installed at each location for
114 recording climatic data every 12 minutes. Next climatic variables were computed based
115 on recorded climatic data: average of daily mean temperature (ºC), mean of daily

116 maximum temperatures (ºC), mean of daily minimum temperatures (ºC), mean of daily
117 relative humidity (%), rainfall (mm), number of days with minimum temperature ≤ 15
118 ºC, number of days with maximum temperature ≥ 30 ºC, number of days with mean
119 temperature ≥ 10 °C and < 15 °C, ≥ 15 and < 20 °C, ≥ 20 and < 25 °C, ≥ 25 and < 30
120 °C, and number of days with rainfall ≥ 2 mm. These climatic variables were selected
121 according to previous reports on the influence of climatic factors on mold development
122 in wheat and maize (Marin et al., 2004, de la Campa et al., 2005, Maiorano et al., 2009,
123 Schaafsma & Hooker, 2007). These parameters were calculated for the next periods: the
124 entire maize growing period, from sowing to harvest; the maize vegetative period, from
125 sowing to silking; the maize reproductive period, from silking to harvest; the flowering
126 period, from 15 days before silking to 15 days after silking; critical period 1 (C1),
127 between 10 and 4 days before silking; critical period 2 (C2), between 4 days before
128 silking and 2 days after silking, critical period 3 (C3), between 2 and 8 days after
129 silking; critical period 4 (C4), between 8 and 14 days after silking; milk-dough kernel
130 stage, between 16 and 30 days after silking; dent kernel stage, between 31 and 45 days
131 after silking; kernel developing period, from silking to physiological maturity; kernel
132 drying period, from physiological maturity to harvest.

133 Other environmental variables included and recorded at harvest were: maize
134 husk coverage, evaluated by a visual scale from 0 (loose husks with visible cob) to 5
135 (tight husks) (Wiseman & Isenhour, 1992); kernel damage by corn borers on a visual
136 rating from 1 (100% of ear totally damaged by borers) to 9 (no damage); tunnel length,
137 maize stem damage by borers expressed in cm; kernel humidity (%); kernel damage by
138 *Sitotroga cerealella*; percentage of kernels with damaged pericarp; and thickness of
139 pericarp expressed in µm.

140 **Identification of *Fusarium* species.** Fifty kernels from each sub-plot were used for
141 estimating the presence of each *Fusarium* species in maize kernels in 2007 and 2008.
142 Maize kernels were grown on KOMADA medium which is selective for *Fusarium* spp.
143 (Komada, 1975). Monosporic isolates were obtained and were grown on PDA (Potato
144 Dextrose Agar), SNA (Spezieller Nährstoffarmer Agar) and CLA (CarnationLeaf Agar)
145 media for determining specific characteristics of each isolate (Leslie & Summerell,
146 2006). In addition, a molecular identification of the species was also performed:

147 Fungal DNA was directly extracted from mycelia of monosporic cultures grown
148 on plates, using the commercial kit E.Z.N.A.® Fungal DNA Mini (Omega bio-tek). All
149 monosporic isolates were tested by PCR. PCR reactions were carried out with primers
150 ITS1 and ITS4 (White et al., 1990) to amplify the ITS region of rDNA, and with
151 primers EF1 and EF2 (O'Donnell et al., 2000) for the elongation factor 1α gene (EF-
152 1α). ITS-PCR reactions were carried out in microcentrifuge tubes each containing one
153 PuReTaq^{M} Ready-To-Go™ PCR Bead (GE Healthcare), 1 µL genomic DNA, 0.3 µL of
154 each primer (10 µM), and sterile water up to a final volume of 25 µL. Elongation factor
155 1α gene PCR-reaction contained 1 µL of genomic DNA, 25 pmol of each primer, 200
156 µL of dNTPs, 1U of Green Taq DNA polymerase (GenScript, USA), 1X standard PCR
157 buffer and sterile water up to a final volume of 25 µL.

158 Both DNA amplification reactions were carried out in a Thermocycler Biometra
159 T3000 (Whatman) under the following conditions: one cycle at 94°C for 5 min; 35
160 cycles at 94°C for 30 s, 55ºC (for ITS1/ITS4) or 53ºC (for EF1/EF2) for 30 s, 72°C for
161 1 min; and a elongation final at 72°C for 10 min. Products from PCR reactions were
162 electrophoresed on a 2% agarose gel, then stained with ethidium bromide, and
163 visualized with a UV transilluminator. The size of PCR products was estimated by
164 comparison with a 100 bp standard ladder (Marker XIV, Roche Diagnostics). Amplified

165 products were sequenced with the same primers used for PCR reactions in an ABIPrism
166 3130 Genetic Analyzer (Applied Biosystems). Sequences obtained were analyzed with
167 the BLAST alignment program of the NCBI and comparing with those deposited in
168 GenBank [National Center for Biotechnology Information (NCIB), 2012]. The
169 molecular identification of a species was accepted when the percentage of sequence
170 identity was above 98%.

171 **Statistical analyses.** The averaged percentage of presence of each *Fusarium* species
172 at each of the 24 environments (combination of 2 years-3 locations-2 sowing dates-2
173 harvest dates) was computed as the mean of individual percentages in 18 sub-plots (six
174 different maize hybrids replicated three times). Combined analyses of variance
175 (ANOVA) for *Fusarium* spp. occurrence were computed with the GLM procedure of
176 SAS following a split-plot design (SAS 2007). All sources of variation were considered
177 as fixed factors. Comparisons of means among years, locations, sowing dates and
178 harvest dates were made by Fisher's protected least significant difference (LSD). In
179 addition, Pearson correlations analyses between *Fusarium* spp. were calculated.

180 In order to examine the relationships between the environmental variables and
181 the *Fusarium* species in the kernels a redundancy analyses (RDA) was performed using
182 CANOCO (Ter Braak & Smilauer, 1997). Previously, a detrented correspondence
183 analysis (DCA) had been performed to determine if data could fit a linear ordination
184 model as RDA or not, following recommendations by Lepš and Šmilauer (2003).
185 Analyses were applied to the averaged percentage of presence of each *Fusarium* species
186 in maize kernels at each environment. RDA computations were performed on centered
187 and standardized data, and run with a forward selection of the environmental variables
188 procedure and the associated Monte Carlo permutation test (499 unrestricted

189 permutations) to exclude environmental variables that did not contribute significantly

190 (p>0.05) to the variation of the *Fusarium* species.

191 **Results**

192 Nine different *Fusarium* species were isolated from maize kernel samples (Table 1).
193 Five species were found in all locations: *F. verticillioides*, complex *F. subglutinans*
194 *sensu lato*, *F. proliferatum*, *F. poae* and *F. oxysporum*. The prevalent species in the 24
195 environments was *F. verticillioides*; the environmental average of *F. verticillioides*
196 presence ranged from 33 to 99 %. The second most abundant was the complex, *F.*
197 *subglutinans sensu lato,* which was present in all environments at percentages varying
198 from 1 to 27 %. The species identified and also included in this complex were *F.*
199 *begoniae* and *F. sterilihyphosum*. The remaining *Fusarium* species (*F. proliferatum*, *F.*
200 *poae*, *F. oxysporum, F. cerealis, F. equiseti, F. solani,* and *F. culmorum*) were present
201 sporadically across environments and never surpassed a kernel presence of 4 % (data
202 not shown).

203 There were no differences between years, locations, sowing dates or harvest
204 dates for the diverse *Fusarium* species identified with the exception of *F. verticillioides*.
205 *F. verticillioides* presence was higher in coastal locations (Pontevedra and Barrantes)
206 compared to the inland location (Valongo). In addition, early sowing (86.19 % early
207 sowing vs. 74.55 % late sowing) and late harvest (73.52 % early harvests vs. 80.94 %
208 late harvests) showed the highest occurrence. No significant differences between years
209 were observed for *F. verticillioides* presence.

210 There was simple positive correlation among abundances for *F. oxysporum* and
211 *F. solani* ($r = 0.67$, $P \leq 0.001$), *F. cerealis* and *F. poae* ($r = 0.56$, $P \leq 0.01$), as well as
212 *F. equiseti* and *F.culmorum* ($r = 0.77$, $P \leq 0.001$), *F. equiseti* and *F. subglutinans sensu*
213 *lato* ($r = 0.59$, $P \leq 0.01$), and *F. culmorum* and *F. subglutinans sensu lato* ($r = 0.70$, $P \leq$
214 0.001). It is important to note that these correlations are based on very low percentages
215 of presence for those species.

The redundancy analysis was performed using significant non-categorical environmental factors as explicative variables. The results of the Monte Carlo permutation tests revealed the statistical significance ($p \leq 0.05$) of the effects of three environmental variables on *Fusarium* species composition: number of days with mean temperature ≥ 15 and < 20 °C during drying kernel period, averaged relative humidity at C3 (between 2 and 8 days after silking), and number of days with minimum temperature ≤ 15 ºC at dent kernel stage (Table 2). The first two axes of the redundancy analysis using these three environmental variables as explicative variables explained the 71.2 % of the variability for *Fusarium* species ocurrence (Figure 1), the 75.0 % of the variability for *F. verticillioides* and 49.0 % of the variability for *F. subglutinans sensu lato* presence (Table 3). Days with mean temperature ≥ 15 and < 20 °C at drying kernel period and days with minimum temperature ≤ 15 ºC at dent kernel stage had an important contribution to the gradient for the first axis which explained the 75 % of variability for *F. verticillioides* (Table 3). The averaged relative humidity during C3 period (between 2 and 8 days after silking) and days with mean temperature ≥ 15 and $<$ 20 °C at drying kernel period had an important effect on the second axis. Both the axes explained 49 % of variability for *F. subglutinans sensu lato* and between 6 and 21% of variability for *F. poae*, *F. proliferatum*, *F. oxysporum*, *F. cerealis*, *F. equiseti, F. solani* and *F. colmorum* (Table 3). Increased days with mean temperature 15 ºC $\leq$ and $<$ 20 ºC at drying kernel period and fewer days with minimum temperature ≤ 15 ºC at dent kernel stage favored the occurrence of *F. verticillioides* in maize kernels (Figure 1); while the presence of *F. subglutinans* augmented with increased relative humidity at C3 period and fewer days with mean temperature 15 ºC $\leq$ and$<$ 20 ºC during kernel drying (Figure 1).

240 **Discussion**

241 All species isolated from maize kernel samples were previously found in maize
242 grown in Europe (Dorn et al., 2009, Goertz et al., 2010, Logrieco et al., 2002). These
243 *Fusarium* species are, in general, mycotoxigenic, and produce fumonisins,
244 trichothecenes, zearalenone, moniliformin, beauvericin, enniatins and fusaric acid
245 (Leslie & Summerell, 2006, Logrieco et al., 2003, Jestoi, 2008). The results confirmed
246 that *F. verticillioides* is the prevalent species in Northwestern Spain (Munoz et al.,
247 1990, Butron et al., 2006).

248 *F. verticillioides* is the most frequently isolated species from maize pink ear rot
249 which is commonly observed from southern to central European areas; while the
250 predominant species causing maize red ear rot is *F. graminearum* which is increasingly
251 distributed from central to northern European regions (Logrieco et al., 2002). In warm
252 southern European areas, *F. verticillioides* is associated with *F. proliferatum*, while
253 displacement toward Central Europe increases the presence of *F. subglutinans* in
254 detriment of *F. proliferatum*. In this study, *F. proliferatum* was scarce and *F.*
255 *graminearum* was not present, while *F. verticillioides* was highly predominant and *F.*
256 *subglutinans sensu lato* was the most abundant group in agreement with the trend
257 observed in surveys performed in the last ten years in maize growing areas around the
258 world where *F. verticillioides* associated with *F. subglutinans* are becoming the
259 dominant species (Bottalico, 1998). Non-detected presence of *F. graminearum* could be
260 consequence of early establishment of *F. subglutinans* that may act as a biological
261 control mechanism against invasion by *F. graminearum* (Cooney et al., 2001) and/or the
262 possible competence between *F. verticilliodes* and *F. graminearum* (Marin et al., 2004,
263 Reid et al., 1999). Environmental conditions at Northwestern Spain, mild temperatures
264 along the year and moderate risk of ear damage by corn borers, can be related to the

265 species distribution. Corn borer damage is associated with increased infection by *F.*
266 *subglutinans* and *F. verticillioides* in detriment of infection by *F. graminearum* (Lew et
267 al., 1991). In addition, more extreme temperatures would favor *F. graminearum*
268 (colder) or *F. proliferatum* (warmer) presence (Logrieco et al., 2002).

269 *F. verticillioides* is a fumonisin producer , and *F. subglutinans* produces a range
270 of mycotoxins including moniliformin, fusaproliferin, beauvericin and fumonisin
271 (Jestoi, 2008).The fumonisin producing capacity of the *F.verticillioides* isolates in the
272 area has been noted (Cao, 2013). In addition, previous studies show the risk of
273 fumonisin occurrence in maize kernels in Northwestern Spain (Butrón et al. 2006; Cao
274 et al, 2013). The higher presence of *F. verticillioides* showed up by the results, obtained
275 in a wide range of environments in natural conditions, support the idea that the
276 fumonisin contamination is the main maize-based feed and food safety concern in this
277 area, although emerging mycotoxins such as moniliformin, fusaproliferin and
278 beauvericin should be also taken into account.

279 The influence of the geographical location on the variability of *F. verticillioides*
280 is important as long as climatic conditions vary across locations (Boutigny et al., 2012).
281 *F. verticillioides* presence was higher in coastal locations compared to the inland
282 location as expected because the coastal climate is more temperate. Variation due to
283 years was not significant; in southern European areas minor differences among years for
284 *Fusarium* variability have been reported (Covarelli et al., 2011, Dorn et al., 2009), while
285 important shift from one year to another for *Fusarium* spp. composition have been
286 found in northern European regions (Goertz et al., 2010, Dorn et al., 2009). About the
287 sowing and harvest dates, we corroborate the role of agronomic practices in order to
288 regulate the occurrence of *F. verticillioides* (Blandino et al, 2009), although slight
289 effects in the *Fusarium* presence has been noted in this particular study, probably with

290 no effect in the subsequent fumonisin contamination. The positive correlation among
291 abundances for *F. subglutinans sensu lato*, *F. equiseti* and *F. culmorum*, as well as
292 between *F. cerealis* and *F. poae,* corroborate that these species are adapted to similar
293 environmental conditions, those encountered in central and northern European areas
294 (Logrieco et al., 2002).

295 Efforts are required to understand the epidemiology of the *Fusarium* disease by
296 focusing more precisely on the relationship between environmental variables and the
297 disease-cycle. Temperature must be considered as an environmental factor that
298 influences spore production under field conditions, in addition to humidity (Indira and
299 Muthusubramanian 2004). In the same way, the mycotoxin contamination is affected by
300 climatic factors such as temperature and relative humidity available for pre and / or
301 post-harvest (Paterson & Lima, 2010). Attending to *F. verticillioides*, the two main
302 abiotic factors associated with the its life cycle are temperature and water activity
303 (Marin et al., 2004; Samapundo et al., 2005), they were considered the main factors in
304 modeling fungal development and fumonisin synthesis (Maiorano et al. 2009, De la
305 Campa et al., 2005). Likewise, under the particular environmental conditions of
306 Northwestern Spain we pointed out temperature and humidity in relation to the
307 *Fusarium* spp. occurrence. We conclude that warmer temperatures at later stages of
308 kernel development and kernel drying period favored the presence of *F. verticillioides*
309 in maize kernels; while the presence of *F. subglutinans sensu lato* augmented with
310 increased relative humidity at the stage of exposed fresh silks and cooler temperatures at
311 the kernel drying period. These results agree with the idea that *F. subglutinans* is
312 favored by cooler temperature and more humid conditions (Logrieco et al., 2002, Goertz
313 et al., 2010, , Boutigny et al., 2012) compared to *F. proliferatum* and *F. verticillioides*.

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435 **Table 1.** Averaged percentages of kernels with presence of *Fusarium* spp. isolates in
436 2007 and 2008 at three locations in Northwestern Spain. The numbers of positive
437 samples are within parenthesis.

***Fusarium* spp.**	**2007**	**2008**
F. verticillioides	75.75 (196)	78.69 (197)
F. subglutinans sensu lato	4.64 (45)	10.34 (85)
F. poae	1.01 (20)	0.07 (2)
F. proliferatum	0.78 (4)	0.05 (1)
F. oxysporum	0.07 (2)	0.96 (11)
F. cerealis	0.15 (1)	0.05 (2)
F. equiseti	0.00	0.17 (4)
F. solani	0.00	0.05 (2)
F. culmorum	0.00	0.10 (2)
Total % of positive kernels	82.40	90.49
Total % of negative kernels	17.60	9.51

438 **Table 2.** Statistics of the environmental variables retained after the Monte Carlo
439 permutation test and included in the RDA for *Fusarium* species composition in maize
440 kernels cultivated in 24 environments (two years, three locations, two sowing dates and
441 two harvest dates) in Northwestern Spain.

Variables[1]	*F*	*p*	Cumulative variance
Tm15-20S	15,87	0,002	0,42
HumC3	12,14	0,002	0,63
Tmin15D	5,65	0,016	0,71

442 [1]Tm15-25S: number of days with mean temperature ≥ 15 ºC and < 20 ºC at the kernel
443 drying period; HumC3: relative humidity at the critical period C3 (between 2 and 8 days
444 after maize silking); Tmin15D: number of days with minimum temperature ≤ 15 ºC at
445 the maize kernel dent stage.

446 **Table 3.** Accumulated variability for each *Fusarium* species abundance at 24
447 environments (two years, three locations, two sowing dates and two harvest dates) in
448 Northwestern Spain explained by three selected significant variables (days with mean
449 temperature ≥ 15 ºC and < 20 ºC at the kernel drying period, relative humidity at the
450 critical period C3 (between 2 and 8 days after maize silking), and days with minimum
451 temperature ≤ 15 ºC at the maize kernel dent stage).

Variability explained	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
F. verticillioides	0.75	0.75	0.75	0.99
F. subglutinans sensu lato	0.01	0.49	0.49	0.65
F. poae	0.01	0.15	0.32	0.32
F. proliferatum	0.06	0.06	0.06	0.09
F. oxysporum	0.05	0.14	0.16	0.16
F. cerealis	0.10	0.17	0.17	0.19
F. equiseti	0.01	0.10	0.12	0.28
F. solani	0.02	0.18	0.19	0.19
F. culmorum	0.08	0.21	0.21	0.31

452 **Figure 1.** Redundancy analysis (RDA) of variability for *Fusarium* species[1] presence

453 restricted to the variability explained by three environmental variables[2].

454 [1]Each *Fusarium* species was designated using the initial of the genera (F) and the initial

455 letters of the Latin specific name: *Fver* stands for *F. verticillioides*, *Fsub_sl* for *F.*

456 *subglutinans sensu lato*, *Fpro* for *F. proliferatum*, *Fcul* for *F. culmorum*, *Fequ* for *F.*

457 *equiseti*, *Fpoa* for *F. poae*, *Foxy* for *F. oxysporum*, *Fsol* for *F. solani*, and *Fcer* for *F.*

458 *cerealis*.

459 [2]Tm15-20S = Mean temperature ≥ 15 ºC and < 20 ºC at the kernel drying period;

460 HumC3 = relative humidity at the critical period 3 (between 2 and 8 days after maize

461 silking); and Tmin15D = number of days with minimum temperature ≤ 15 ºC at the

462 maize kernel dent stage.