

1 **Mycotoxins in maize grains grown in organic and conventional agriculture**

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12

13 **Abstract**

14 Maize is traditionally used for bakery in several countries, and autochthonous varieties are  
15 increasingly demanded particularly for organic agriculture, but one of the dangers of cereal  
16 consumption is mycotoxin contamination. Mycotoxins are dangerous for health and might be  
17 present in any grain depending on genotypes and environments. In the present work we assess  
18 the natural levels of fumonisin and deoxynivalenol (DON) contaminations in nine diverse open-  
19 pollinated maize varieties grown in four different locations, under organic or conventional  
20 conditions, in two regions from the humid Spain during two years. Differences were significant  
21 among locations and among varieties for fumonisin contamination but not for DON content.  
22 Locations were the main environmental source of variation affecting fumonisins while DON  
23 was more affected by years. The Basque locations had more fumonisin than the Galician  
24 locations, but there were no differences between organic and conventional environments.  
25 Fumonisin contamination was more variable than DON among locations and among varieties.  
26 Fumonisin and DON were highly correlated on average but correlations were low for each  
27 particular environment. Mean fumonisin and DON were below the threshold allowed by the  
28 EU, but the white-kernel medium late variety Rebordanes(P)C2 had more than 4.00 mg/kg of  
29 fumonisin in one location, while the early yellow variety Sarreaus had the lowest  
30 contamination. We conclude warning producers of the danger of natural contamination with  
31 mycotoxins for some varieties in specific environments.

32

33 **Keywords:** fumonisin, deoxynivalenol, DON, maize, landraces, organic agriculture

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35

## 36 **Introduction**

37

38 Varieties of maize are traditionally used for bakery in northern Spain, Portugal and other  
39 countries (Landa et al., 2006; Revilla et al., 2008; Vaz Patto et al., 2007). Besides quality and  
40 flavor, these traditional varieties are interesting due to their potential value as functional foods  
41 (Rodríguez et al., 2013). Moreover, there is an increasing interest for reintroducing improved  
42 varieties for food, particularly under organic agriculture (Landa et al., 2006; Revilla et al., 2008,  
43 2012). Although the amount of maize used for food is lower than for feed, the economic value  
44 of maize for food is high and poses some health and safety problems, for example, reduced  
45 levels of contaminants are allowed compared with maize. In this context, organic agriculture is  
46 considered safer than conventional agriculture because inorganic fertilizers and phytosanitary  
47 synthetic products are forbidden. No final conclusion about which is the best agricultural  
48 system for reducing the risk of contamination with mycotoxins have been drawn (Ariño et al.,  
49 2007; Magkos et al., 2006; Cirillo et al., 2003).

50 Mycotoxins are produced by several species of fungi, being *Fusarium* the most common  
51 genus in the European Atlantic coast; in southern regions, the most frequent fungus found in  
52 maize grains is *F. verticillioides* Saccardo (= *F. moniliforme* Sheldon) Nirenberg that produces  
53 fumonisins (Logrieco et al., 2003), while in colder regions, *F. graminearum* Schwabe that  
54 produces deoxynivalenol (DON) could be predominant (Hooker and Schaafsma, 2005). DON is  
55 also produced by *F. culmorum* and *F. cerealis* (Marin et al., 2013). The Rapid Alert System for  
56 Food and Feed of the European Union has registered 14 alerts of fumonisins and 24 of DON  
57 between 2008 and 2012 in cereals and bakery products; most of these alerts were for maize  
58 samples (RASSF, 2006). Breakfast cereals and baby foods also contained DON, although at  
59 lower levels than unprocessed maize kernels (Marin et al., 2013). Fumonisins cause diverse  
60 health problems in animals and, in humans, fumonisins could be related with increased

61 incidence of esophageal cancer and neural tube defects and are considered as probably  
62 carcinogenic (Bennett and Klich, 2003; IARC, 1993). There are no data supporting the possible  
63 mutagenic or carcinogenic effects of DON. IARC has included DON in Group 3 and  
64 fumonisins in group 2.4. Although DON is not as toxic as other mycotoxins, it is one of the  
65 most common contaminants of cereals worldwide. Acute effects of food poisoning in humans  
66 are abdominal pains, dizziness, headache, throat irritation, nausea, vomiting, diarrhea, and  
67 bloody stools.

68         During the bakery process, DON is considerably reduced, but fumonisins are fairly heat-  
69 stable as there is little degradation during fermentation and toxin content is significantly  
70 reduced only during processes in which the temperature exceeds 150 °C (Marin et al., 2013).  
71 Therefore, there is an increasing amount of legislation limiting the amount of fumonisins (FAO,  
72 2004) and the European Union establishes that the threshold for fumonisin contents is 4.00  
73 mg/kg and 1.75 mg/kg for DON in non processed maize (UE, 2006, 2007).

74         Mycotoxin contamination depends on the fungi isolate, but also on environmental and  
75 genetic background of the maize crop (Picot et al., 2010; Warfield and Gilchrist, 1999). Poor  
76 agricultural and harvesting practices, improper drying, handling, packaging, storage, and  
77 transport conditions promote fungal growth, increasing the risk of mycotoxin production  
78 (Marin et al., 2013). These authors stated that fumonisins are the most important mycotoxins  
79 found in maize, particularly when grown in warmer regions, as *F. verticillioides* and *F.*  
80 *proliferatum* grow over a wide range of temperatures, but only at relatively high water  
81 activities. Cao et al. (2013) evaluated fungal infection and fumonisin accumulation at different  
82 kernel development stages and during kernel drying in three white maize hybrids and found that  
83 *Fusarium*, especially *F. verticillioides*, was the most prevalent genera compared to *Aspergillus*  
84 and *Penicillium*. Kernel damage by insects and suboptimal temperatures for fungal growth  
85 when kernel humidity is low favored an increased rate of fumonisin accumulation (Cao et al.

86 2013). Cao et al. (2014) also concluded that the fungal growth rate significantly increased with  
87 temperature and water activity and found variability for genetic resistance to fungal infection  
88 and fumonisins accumulation.

89 Several authors have searched variability for resistance to fumonisin contamination  
90 among maize inbreds or hybrids. For example, Clements et al. (2004) evaluated a collection of  
91 inbred lines crossed to a tester under artificial infestation with *Fusarium verticillioides* and  
92 found significant differences for fumonisin contamination. Similarly, Afolabi et al. (2007)  
93 evaluated a collection of maize inbred lines per se under natural infestation and found  
94 differences among maize inbreds and genotype × environment interaction for fumonisin  
95 contamination as well as positive correlation between fungi infection and fumonisin  
96 contamination only in one of the two Nigerian localities used. Presello et al. (2007) evaluated a  
97 group of maize hybrids and found positive correlations between symptom severity and  
98 concentration of fumonisins, indicating that genotypic effects for concentration of fumonisins  
99 in grain mainly depended on genotypic effects for disease resistance. Löffler et al. (2010)  
100 analyzed correlations between mycotoxin concentrations and ear rot rating of 50 inbred lines  
101 under artificial infestation and found that the early maturity group flint lines were more  
102 susceptible and there were broad ranges and significant genotypic variances as well as genotype  
103 x environment interaction variances, but also high heritabilities for ear rot and mycotoxin  
104 concentrations. Henry et al. (2009) evaluated a selected group of inbred lines inoculated with  
105 either *A. flavus* or *F. verticillioides* and found significant variability for resistance to aflatoxin  
106 and fumonisin contamination among maize inbred lines and inbreds resistant to aflatoxin were  
107 also resistant to fumonisin contamination. Santiago et al. (2013) evaluated 240 maize inbred  
108 lines under kernel inoculation with *Fusarium verticillioides* and found differences for resistance  
109 to fumonisin contamination across environments. Bolduan et al. (2009) evaluated in Germany a  
110 collection of maize inbreds for mycotoxin contamination, including DON, and found significant

111 genotypic and genotype  $\times$  environment interaction variances, moderate heritabilities, and high  
112 correlations between disease severity and mycotoxin concentrations. DON has been found in  
113 naturally infected ears across nine locations in Germany (Magg et al., 2002). Relatively high  
114 contaminations of DON were reported in maize genotypes by Hart et al. (1984).

115         Even though it is clear that there are differences among locations and genotypes for  
116 resistance to fumonisin contamination, as far as we know, no previous report has been  
117 published comparing contamination levels in different open-pollinated maize varieties and in  
118 different locations. In the present work we assess the levels of mycotoxin contamination in nine  
119 diverse open-pollinated maize varieties with different grain colors grown in four different  
120 locations, under organic or conventional conditions, in two regions from northern Spain.

121

122 **Materials and Methods**

123

124 We evaluated nine open-pollinated maize varieties in two farmers' fields in Galicia and in the  
125 Basque Country under organic and conventional agriculture in 2010 and 2011. Among the  
126 varieties evaluated there was one with early cycle (Sarreaus(P)C2), six with medium cycle  
127 (Carballeira, DonostiaC1, Martikoenea, Oroso, Tuy(S)C3 and Txalin) and two with medium-  
128 late cycle (Meiro(P)C2 and Rebordanes(P)C2). Concerning grain color, six varieties had yellow  
129 endosperm and three had white endosperm, one of them with transparent pericarp  
130 (Rebordanes(P)C2) and two with black pericarp (Carballeira and Meiro(P)C2). The locations  
131 included two organic fields [Lobeira, 600 masl (Galicia) and Heredia , 567 masl (Basque  
132 Country)] and two conventional fields [Pontevedra , 20 m masl (Galicia) and Arkaute, 550 masl  
133 (Basque Country)].

134 The varieties were evaluated following a randomized complete block design with three  
135 replications. The experimental plots of 10 m<sup>2</sup> had a density of 60,000 plants ha<sup>-1</sup>, with rows  
136 separated 0.8 m and plants within rows 0.21 m. Agricultural practices followed the  
137 recommendations of organic agriculture, i.e. nutrients were supplied by adding manure, weeds  
138 were removed mechanically, and no chemical treatment was used. Under conventional  
139 agriculture, current practices were the usual in the area with inorganic fertilizers, use of  
140 herbicide and no irrigation.

141 Ears from each plot were collected when grains were dry. A representative kernel  
142 sample of approximately 200 g was ground and the resulting flour sample was maintained at 4  
143 °C until performing chemical analyses. Kernels were ground through a 0.75 mm screen in a  
144 Pulverisette 14 rotor mill (Fritsch GmbH, Oberstein, Germany). We have performed three  
145 replicates of each sample. Mycotoxins were determined using the commercial kit Veratox  
146 (Neogen Corp., Lansing MI) a competitive direct ELISA (CD-ELISA) that provides a

147 quantitative analysis of fumonisins and deoxynivalenol (DON) with a lower limit of detection  
148 of 0.1 ppm. The kit has the ISO 9001 certificate and the controls provided were 0, 1, 2, 4 and 6  
149 ppm for fumonisine and 0, 0.5, 1, 2 and 6 ppm for DON. The recovery rate was over 90% in all  
150 samples (Neogen Corp. Lansing MI).

151 Free fumonisin and DON in the samples and controls is allowed to compete with  
152 enzyme-labeled fumonisin and DON (conjugate) for the antibody binding sites. After a wash  
153 step substrate is added, which reacts with the bound conjugate to produce blue color. The  
154 antibody specific cross reactivity was total fumonisins and DON 100%. The color of the test  
155 line was compared with the test line of a negative control strip. Readings were performed with a  
156 spectrophotometer at 650 nm.

157 Analyses of variance were carried out using the procedure GLM of SAS (SAS Institute  
158 Inc. 2010) with two years and four locations. Two locations were under conventional conditions  
159 and two under organic conditions. First, we made combined analyses of variance over years and  
160 locations in order to check the genotype  $\times$  environment interaction (GE); locations, varieties  
161 and their interaction were considered fixed effects and the other sources of variation were  
162 random. Further analyses of variance were carried out considering each year-location  
163 combination as one environment; varieties were the only fixed effect in these analyses.  
164 Comparisons of means were made by using the Fishers' protected LSD at  $P=0.05$ . Simple  
165 correlations between fumonisin and DON were calculated with the procedure CORR of SAS  
166 (SAS Institute Inc. 2010).

167



168 **Results and discussion**

169 Analyses of variance over years and locations showed significant differences among  
170 locations and among varieties for fumonisin contamination and the location  $\times$  variety  
171 interaction was significant for fumonisin. Similarly, several authors have found significant  
172 differences among inbred lines and significant genotype  $\times$  environment interaction for  
173 fumonisin content (Clements et al. 2004, Afolabi et al., 2007, Löffler et al., 2010, Henry et al.,  
174 2009, Santiago et al., 2013). Differences were not significant among open-pollinated varieties  
175 for DON and the year  $\times$  variety interaction was significant for DON. However, under artificial  
176 infection, previous reports have found significant differences among maize inbred lines and  
177 significant genotype  $\times$  environment interaction for DON contamination (Bolduan et al., 2009,  
178 Hart et al., 1984). Most interactions were of magnitude but some of them were of rank.  
179 Similarly, several authors have reported genotypic stability of genotypes for mycotoxin  
180 contamination when field trials were done in a wide range of environments (Robertson et al.,  
181 2006, Hung and Holland, 2012, Presello et al., 2006). The significant GE effects have been  
182 attributed to heterogeneity of genotypic variance, rather than to the lack of correlation of  
183 genotype performance at different environments (Robertson et al., 2006).

184 The levels of fumonisin contamination in both environments of the Basque Country  
185 (Arkaute and Heredia) were more than five times higher than the levels of fumonisin in the  
186 Galician environments (Pontevedra and Lobeira) (Table 1a). The environments with organic  
187 agriculture (Heredia and Lobeira) were not significantly different for fumonisin contamination  
188 from those with conventional agriculture (Arkaute and Pontevedra). Accordingly, other authors  
189 have reported that the farming system is probably not of decisive importance for the final  
190 contamination of agricultural products with these mycotoxins (Ariño et al., 2007; Magkos et al.,  
191 2006; Cirillo et al., 2003). Although differences among locations for DON were not significant,  
192 they followed a pattern of variation similar to that for fumonisin content.

193           When each combination year-location was considered a different environment,  
194 differences among environments were significant for both fumonisin and DON contents (Table  
195 1a). The range of variation was larger for fumonisin (0.15 to 1.76) than for DON (0.03 to 0.73),  
196 although none of the values reached the threshold established by the European Union for  
197 mycotoxins (EU, 2006, 2007). The Basque environments had the highest fumonisin content,  
198 although Pontevedra 2011 was not significantly different from the Basque locations in 2010.  
199 Arkaute 2011 and Heredia 2010 had the highest DON contamination, followed by Arkaute  
200 2010 that was not significantly different from the Galician locations in 2011. The correlation  
201 between fumonisin and DON content was very high and significant ( $r^2 = 0.92$   $P < 0.01$ ) when the  
202 correlation was calculated with the means of mycotoxin for varieties content across years and  
203 locations. When the correlation between fumonisins and DON was calculated for each  
204 environment, correlations were low and not significant. Therefore, even though varieties with  
205 higher fumonisin content had also higher DON content on average, this relationship was not  
206 consistent across environments. Our results indicate that locations were more important in the  
207 genotype  $\times$  environment interactions for fumonisin content while yearly variation was more  
208 important for DON.

209           In the combined analyses of variance, the varieties with higher fumonisin content were  
210 Rebordanes(P)C2 (white grain), Martikoenea (yellow grain), and the black-kernel varieties  
211 Meiro(P)C2 and Carballeira with white endosperm (Table 1b). The fumonisin content of the  
212 low black-kernel varieties was not significantly different from the yellow varieties with less  
213 fumonisin content. The varieties with fumonisin contamination significantly below  
214 Rebordanes(P)C2 were all yellow. Santiago et al. (2013) also found that white-grain maize had  
215 higher fumonisin content than yellow-grain ( $P = 0.06$ ), but many white inbreds were among  
216 those with less contamination. It should be also noted that the varieties with larger growth cycle  
217 (Rebordanes(P)C2 and Meiro(P)C2) were among those with higher fumonisin contamination,

218 while the earliest variety Sarreaus(P)C2 was among those with lowest fumonisin  
219 contamination. These results are consistent with the fumonisin contamination detected at  
220 various stages of development by Cao et al. (2013). Contrarily, Löffler et al. (2010) found that  
221 the earliest genotypes were more susceptible than the latest ones.

222         Given that differences among fumonisin contamination between varieties were  
223 significant under conventional agriculture and not under organic agriculture, the rank of the  
224 varieties in the combined analysis over environments was very similar to that of conventional  
225 agriculture. The means for fumonisin and DON contents at each environment were always  
226 below the threshold of 4.00 mg/kg and 1.75 mg/kg, respectively (UE, 2006, 2007). However,  
227 when looking at the mean of each variety in each environment, Rebordanes (P)C2 had 4.07  
228 mg/kg of fumonisin in Arkaute 2011 (Table 2). Furthermore, Martikoenea in Pontevedra 2011,  
229 Rebordanes(P)C2 in Arkaute 2010 and Meiro(P)C2 in Arkaute 2011 had fumonisin contents  
230 close to that threshold. The DON contamination, was always below 1.75, although Oroso in  
231 Pontevedra 2011 and Rebordanes(P)C2 in Lobeira 2011 were close to that value. Several  
232 authors have hypothesized that the competence between *F. verticillioides* and *F. graminearum*  
233 limit the presence of *F. graminearum*. (Marin et al., 2004, Butrón et al., 2006, Munoz et al.,  
234 1990). Although in this study we do not measure fungi, some authors have shown that fungi  
235 infection and fumonisin contamination are positively correlated (Afolabi et al., 2007, Presello et  
236 al., 2007, Santiago et al. 2013). The previous results of fungal infection are consistent with the  
237 higher contamination of fumonisins compared to DON found here.

238         Even though fumonisin was higher than DON contamination, fumonisin content was  
239 zero in 25% of the cases while DON was zero only in 3% of the cases (Table 3). Furthermore,  
240 the range of fumonisin content was larger than that of DON as in each location at least one  
241 variety had 0 mg/kg of fumonisin and the most contaminated variety had from 0.46 to 4.07  
242 mg/kg. These results were obtained under natural contamination, but, under artificial

243 infestation, *F. verticillioides* attack was consistent across environments (Santiago et al. 2013).  
244 All varieties had appreciable levels of DON, except Meiro(P)C2 and Rebordanes(P)C2 in  
245 Pontevedra 2010. Therefore, attention should be paid to mycotoxin contamination when  
246 specific varieties are grown in some locations and years because mycotoxins are almost always  
247 present and the amount could rise above the safety levels in some years and locations.

248

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348



349 Table 1. Mycotoxin content (ppm) in maize grain from 9 varieties evaluated 2 years in 4  
 350 locations

351 a) Means by locations

Locations	Fumonisin			DON		
	Combinad	2010	2011	Combinado	2010	2011
	o					
Arkaute	1.48 a	1.22	1.76	0.60	0.58	0.82
Heredia	1.42 a	1.42	-	0.45	0.73	-
Pontevedra	0.52 b	0.21	0.83	0.38	0.16	0.45
Lobeira	0.21 b	0.15	0.27	0.38	0.03	0.45
LSD (0.05)	0.72	0.92 <sup>a</sup>		0.17 <sup>a</sup>		

<sup>a</sup> LSD calculated for comparisons among environments considering each

environment as the combination of one year and one location

352 b) Fumonisin content (ppm) by varieties in conventional and organic culture.

	Combined	Conventional	Organic		
Rebordanes(P)C	1.87 a	Martikoenea	2.40 a	Rebordanes(P)C	1.25
2				2	
Martikoenea	1.74 ab	Rebordanes(P)C	2.33 a	Carballeira	0.98
		2			
Meiro(P)C2	1.04 abc	Meiro(P)C2	1.54 ab	Txalín	0.96
Carballeira	1.00 abc	Carballeira	1.02	Martikoenea	0.85
			abc		
Tuy(S)C3	0.80 bc	Tuy(S)C3	0.79 bc	Tuy(S)C3	0.82
Txalín	0.48 c	Osoro	0.51 bc	Meiro(P)C2	0.39
Osoro	0.33 c	Sarreus(P)C2	0.18 bc	DonostiaC1	0.12
Sarreus(P)C2	0.13 c	Txalín	0.12 c	Osoro	0.08
DonostiaC1	0.11 c	DonostiaC1	0.11 c	Sarreus(P)C2	0.08
LSD (0.05)	0.94		1.39		
Media	0.83		1.00		0.61

353

354 Table 2. Fumonisin content (ppm) in maize grain of nine open-pollinated populations evaluated in seven environments <sup>a</sup>.

355	Environments		1	2	3	4	5	6	7							
356		Rep	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev
357	Txalín	1	3.211	0.0020.000	0.000	0.102	0.008	0.000	0.000	0.341	0.011	0.000	0.000	0.000	0.000	
358	Sarreus(P)C2	1	0.000	0.0000.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.398	0.017	0.000	0.000	
359	Meiro(P)C2	1	0.000	0.0002.623	0.005	3.915	0.009	0.000	0.000	0.608	0.018	0.366	0.016	0.491	0.019	
360	DonostiaC1	1	0.000	0.0000.000	0.000	1.166	0.009	0.000	0.000	0.000	0.000	0.436	0.016	0.000	0.000	
361	Tuy(S)C3	1	0.000	0.0000.000	0.000	0.911	0.008	0.000	0.000	0.373	0.012	0.385	0.015	0.000	0.000	
362	Osoro	1	0.000	0.0000.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.451	0.016	3.168	0.015	
363	Rebordanes(P)C2	1	0.709	0.0030.000	0.000	1.250	0.011	3.669	0.067	0.646	0.011	0.385	0.021	1.875	0.013	
364	Carballeira	1	0.000	0.0004.544	0.006	6.667	0.008	0.000	0.000	0.629	0.015	0.967	0.016	0.000	0.000	
365	Martikoenea	1	0.000	0.0008.528	0.008	8.721	0.008	0.000	0.000	0.000	0.000	0.273	0.012	0.000	0.000	
366	Txalín	2	0.000	0.0000.000	0.000	0.000	0.000	0.000	0.000	0.352	0.014	0.000	0.000	0.000	0.000	
367	Sarreus(P)C2	2	0.000	0.0000.000	0.000	0.000	0.000	0.000	0.000	0.526	0.014	0.230	0.017	1.581	0.013	
368	Meiro(P)C2	2	0.287	0.0024.250	0.010	4.638	0.003	0.000	0.000	0.545	0.016	0.366	0.021	0.000	0.000	
369	DonostiaC1	2	0.000	0.0000.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.304	0.013	0.000	0.000	
370	Tuy(S)C3	2	0.894	0.0030.743	0.006	3.481	0.010	0.000	0.000	0.238	0.020	0.364	0.014	0.000	0.000	

371	Osoro	2	0.000	0.0000.000	0.000	0.000	0.000	0.000	0.000	0.000	0.459	0.017	0.000	0.000	0.000	0.000
372	Rebordanes(P)C2	2	6.012	0.0047.467	0.008	7.666	0.009	0.000	0.000	0.000	0.000	0.000	0.408	0.015	0.773	0.012
373	Carballeira	2	5.178	0.0090.000	0.000	0.342	0.008	0.000	0.000	0.000	0.000	0.000	0.418	0.019	0.000	0.000
374	Martikoenea	2	7.200	0.0050.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6.826	0.013
375	Txalín	3	5.414	0.0060.000	0.000	0.014	0.002	0.000	0.000	0.646	0.017	0.000	0.000	0.000	0.000	0.000
376	Sarreaus(P)C2	3	0.000	0.0000.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.013	0.000	0.000
377	Meiro(P)C2	3	1.762	0.0050.320	0.005	0.923	0.006	0.331	0.013	0.117	0.015	0.359	0.017	0.000	0.000	0.000
378	DonostiaC1	3	0.000	0.0000.114	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.345	0.017	0.000	0.000
379	Tuy(S)C3	3	5.538	0.0031.851	0.008	1.862	0.009	0.000	0.000	0.000	0.000	0.157	0.014	0.000	0.000	0.000
380	Osoro	3	0.000	0.0000.045	0.005	0.828	0.010	0.000	0.000	0.000	0.000	0.303	0.019	1.603	0.012	0.000
381	Rebordanes(P)C2	3	0.000	0.0003.627	0.003	3.283	0.008	0.000	0.000	0.000	0.000	0.079	0.022	1.396	0.019	0.000
382	Carballeira	3	2.247	0.0020.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
383	Martikoenea	3	0.000	0.0000.000	0.000	0.000	0.000	0.000	0.000	0.090	0.011	0.189	0.013	4.649	0.016	0.000

384 <sup>a</sup> 1=Heredia 2010, 2=Arkaute 2010, 3=Arkaute 2011, 4=Lobeira 2010, 5=Pontevedra 2010, 6=Lobeira 2011, 7=Pontevedra 2011

385

386 Table 3. Deoxynivalenol (DON) content (ppm) in maize grain of nine open-pollinated populations evaluated in seven environments<sup>a</sup>.

Environments	1		2		3		4		5		6		7		
	Re p	Mea n	StdDev	Mean	StdDev	Me an	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev
Txalín	1	1.21				0.2									
		0	0.008	0.500	0.013	40	0.013	0.020	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Sarreus(P)C2	1	0.94				0.9									
		0	0.009	1.190	0.013	80	0.013	1.310	0.013	0.000	0.000	0.159	0.010	0.000	0.000
Meiro(P)C2	1	1.25				0.6									
		0	0.012	0.880	0.013	00	0.009	0.840	0.013	0.000	0.000	0.166	0.014	1.110	0.108
DonostiaC1	1	0.80				0.2									
		0	0.013	0.540	0.011	62	0.012	0.680	0.011	0.000	0.000	0.110	0.009	0.000	0.000
Tuy(S)C3	1	0.83				1.1									
		0	0.009	1.440	0.007	30	0.013	1.070	0.012	0.000	0.000	0.100	0.005	1.263	0.012
Osoro	1	0.11				0.6									
		0	0.005	0.970	0.013	50	0.011	0.570	0.015	0.080	0.012	0.140	0.010	1.470	0.006
Rebordanes(P)C2	1	1.04				0.4									
		0	0.014	0.770	0.080	10	0.015	0.650	0.014	0.000	0.000	0.160	0.009	0.957	0.008
Carballeira	1	0.09				0.4									
		0	0.006	0.760	0.008	00	0.011	0.000	0.000	0.036	0.010	0.140	0.012	0.406	0.006
Martikoenea	1	0.30				0.3									
		0	0.006	0.690	0.009	40	0.012	0.000	0.000	0.020	0.005	0.220	0.013	0.592	0.009

Txalín	2	0.07				0.3								
		0	0.008	0.630	0.010	10	0.013	0.000	0.000	0.000	0.012	0.000	0.000	0.000
Sarreus(P)C2	2	0.66				0.3								
		0	0.008	0.620	0.009	00	0.013	0.330	0.013	0.554	0.005	0.150	0.013	0.499
Meiro(P)C2	2	0.46				0.3								
		0	0.005	0.640	0.010	10	0.018	0.510	0.013	0.000	0.012	0.230	0.020	0.827
DonostiaC1	2	0.22				0.2								
		0	0.009	0.480	0.009	00	0.010	0.000	0.000	0.035	0.010	0.350	0.013	0.488
Tuy(S)C3	2	0.90				0.4								
		0	0.044	0.710	0.015	80	0.009	0.750	0.014	0.000	0.000	0.000	0.000	0.000
Osoro	2	0.11				0.2								
		0	0.009	0.530	0.009	60	0.013	0.990	0.009	0.000	0.000	0.150	0.008	0.000
Rebordanes(P)C2	2	0.00				0.5								
		0	0.000	0.810	0.013	10	0.018	0.900	0.027	0.000	0.000	0.350	0.007	0.986
Carballeira	2	0.86				0.4								
		0	0.013	0.730	0.008	90	0.010	0.530	0.014	0.000	0.000	0.184	0.010	0.000
Martikoenea	2	0.61				0.3								
		0	0.009	0.600	0.014	00	0.012	1.000	0.015	0.010	0.004	0.290	0.009	0.550
Txalín	3	0.13				0.7								
		0	0.006	1.070	0.005	10	0.022	1.250	0.017	0.002	0.002	0.000	0.000	0.080
Sarreus(P)C2	3	0.00				0.5								
		0	0.000	0.820	0.016	10	0.021	0.650	0.014	0.035	0.006	0.240	0.014	0.550

Meiro(P)C2	3	0.11				0.3									
		0	0.004	0.630	0.009	30	0.011	0.940	0.015	0.000	0.000	0.000	0.000	0.622	0.021
DonostiaC1	3	0.13				0.4									
		0	0.007	0.690	0.008	00	0.015	1.000	0.009	0.010	0.002	0.230	0.016	0.270	0.014
Tuy(S)C3	3	0.39				0.3									
		0	0.009	0.690	0.013	40	0.010	0.039	0.016	0.025	0.006	0.000	0.000	0.392	0.007
Osoro	3	0.00				0.2									
		0	0.000	0.590	0.014	30	0.018	0.800	0.015	0.000	0.000	0.230	0.015	1.980	0.016
Rebordanes(P)C2	3	0.28				0.3									
		0	0.014	0.710	0.037	50	0.011	0.040	0.016	0.000	0.000	0.250	0.009	1.800	0.017
Carballeira	3	0.59				0.5									
		0	0.026	0.830	0.013	60	0.014	0.350	0.015	0.000	0.000	0.000	0.000	1.180	0.018
Martikoenea	3	0.14				0.4									
		0	0.008	0.770	0.014	60	0.012	0.410	0.013	0.000	0.000	0.000	0.000	1.220	0.013

387 <sup>a</sup> 1=Heredia 2010, 2=Arkaute 2010, 3=Arkaute 2011, 4=Lobeira 2010, 5=Pontevedra 2010, 6=Lobeira 2011, 7=Pontevedra 2011

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