

1 *Field Crops Research*

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5 **Assessment of corn resistance to fumonisin accumulation in a broad collection of**
6 **inbred lines**

7 Rogelio Santiago¹, Ana Cao¹, Rosa A Malvar¹, Lana M Reid², Ana Butrón^{1*}

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11 ¹ Misión Biológica de Galicia (CSIC), Apdo. 28, 36080 Pontevedra, Spain.

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13 ² Eastern Cereal and Oilseed Research Centre, Central Experimental Farm, Agriculture
14 and Agri-Food Canada, Ottawa, Ontario, Canada, K1A 0C6

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17

18 * For correspondence: E-mail rsantiago@mbg.csic.es; Telephone +34 986 854800; Fax

19 +34 986841362

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23 **Abstract**

24 Genetic improvement is an effective and environmentally safe method to reduce the
25 levels of fumonisin mycotoxins in corn kernels infected with *Fusarium verticillioides*.
26 In order to find new sources of resistance, a wide collection of corn inbred lines were
27 evaluated for Fusarium ear rot and fumonisin accumulation after inoculation of the
28 kernels with *F. verticillioides*. Augmented designs were used for testing 240 un-
29 replicated inbreds and 6 inbred checks in 2010 and 2011. Sixty-one inbreds were found
30 to have the highest levels of resistance to Fusarium ear rot and fumonisin accumulation
31 across years. Inbreds differing in kernel color, use, kernel type and heterotic group were
32 all represented in this group of 61 inbreds. White corn inbreds had higher levels of
33 fumonisin than yellow corn inbreds, but it was still possible to find white inbreds with
34 comparable resistance to fumonisin accumulation to that of the most resistant yellow
35 inbreds. Similarly, although the sweet corn inbreds evaluated in this study were less
36 resistant to infection by *F. verticillioides* than the field corn inbreds, there were some
37 which were grouped in the most resistant 61 inbreds. Many of these inbreds can be used
38 to improve resistance to *F. verticillioides* infection and fumonisin accumulation by
39 crossing the most resistant inbreds of each subgroup.

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42 **KEYWORDS:** *Zea mays*, heterotic group, breeding, *Fusarium verticillioides*,
43 fumonisin accumulation.

44 **1. Introduction**

45 *Fusarium verticillioides* is the most prevalent fungus found on corn in Spain (Butrón et
46 al., 2006) and poses a feed and food safety problem because most *F. verticillioides*
47 isolates are capable of producing the fumonisin mycotoxins (Abarca et al., 2000; de
48 Oliveira Rocha et al., 2011; Tancic et al., 2012). These toxins are accumulated in corn
49 kernels. Fumonisin toxicity is related to a capacity to disrupt the biosynthesis of
50 sphingolipids, the main components of the plasmatic membrane of the cell, resulting in
51 apoptosis and disturbances of cellular processes such as cell growth, and cell
52 differentiation and morphology (SCF, 2000; Voss et al., 2007). In humans, fumonisins
53 are suspected risk factors for esophageal cancer and neural tube defects (Bennett and
54 Klich, 2003). The International Agency for Research on Cancer has classified
55 fumonisins as probable carcinogens (IARC, 1993). In livestock, these toxins cause
56 leukoencephalomalacia in horses, pulmonary edema in pigs, reduced growth in poultry
57 and hepatic and immune disorders in cattle (Logrieco et al., 2003; Voss et al., 2007).
58 Legislation to limit the amount of fumonisins in foods and feedstuffs has been
59 implemented in many parts of the world (FAO, 2004). The European Union established
60 threshold fumonisin contents of 4000 µg/kg in non-processed corn, 1000 µg/kg in corn
61 intended for direct human consumption, 800 µg/kg in corn-based breakfast cereals and
62 snacks, 200 µg/kg in corn-based products for infants and young children, 1400 µg/kg in
63 milling fractions with particle size > 500 µm, 2000 µg/kg in milling fractions with
64 particle size < 500 µm (1126/2007/EC, 2007), and recommends levels below 5000-
65 50000 µg/kg, depending on the animal, for livestock feed (576/2006/EC, 2006).
66 As genetic improvement is emerging as an effective and environmentally safe
67 method to reduce the levels of fumonisins in corn (Eller et al., 2008), the search for
68 sources of resistance has been the focus of many studies (Pascale et al., 2002;

69 [Kleinschmidt et al., 2005](#); [Afolabi et al., 2007](#); [Presello et al., 2007](#); [Henry et al., 2009](#);
70 [Löffler et al. 2010](#)). Kernel and silk channel inoculations mimic kernel infection
71 vectored by insects and by spores deposited on silks by rain or/and wind, respectively.
72 Of these, [Schaafsma et al. \(2006\)](#) found a consistent correlation between Fusarium ear
73 rot and fumonisin accumulation after inoculation by kernel wounding with *F.*
74 *verticillioides* but not with channel inoculation. This study suggests that kernel
75 inoculation would be more suitable inoculation technique to use when screening
76 genotypes for resistance to fumonisin accumulation by using Fusarium ear rot disease
77 severity as an indirect selection criterion. Partial resistance to infection by *F.*
78 *verticillioides* and to the accumulation of fumonisins have been identified in several
79 different types of corn germplasm ([Clements et al., 2004](#); [Afolabi et al., 2007](#); [Presello](#)
80 [et al., 2007](#); [Henry et al., 2009](#); [Löffler et al. 2010](#)); however, until now, an exhaustive
81 evaluation of the germplasm adapted to the environmental conditions of the southern
82 European Atlantic Coast has not been carried out. The inbred collection maintained at
83 the Misión Biológica de Galicia (Spanish Council of Scientific Research) is well
84 adapted to the southern European Atlantic Coast and includes materials developed from
85 Spanish landraces which may supply more rare alleles than other European landraces
86 since Spain was the main entrance of corn to Europe from the Americas ([Revilla et al.,](#)
87 [2003](#)). The purpose of the current study was: 1) to evaluate a collection of inbred lines
88 from the Misión Biológica de Galicia in order to study the relationship between
89 fumonisin accumulation and Fusarium ear rot in this adapted germplasm base; 2) to
90 investigate the influence of kernel characteristics, such as color (white and yellow), type
91 (dent and flint), use (popcorn, sweet corn and field corn), mutation (waxy, opaque), and
92 heterotic group (European, Reid, Lancaster, Northern Flint, Minnesota No 13, other
93 Corn Belt, and miscellaneous) on Fusarium ear rot and fumonisin accumulation; and 3),

94 to identify sources of resistance for incorporation into a breeding program to develop
95 resistance to *F. verticillioides* in this geographical region.

96 **2. Materials and methods**

97 *2.1. Plant material and experimental design*

98 Three hundred entries were evaluated for resistance to *F. verticillioides* at Pontevedra,
99 Spain (42°24' N, 8°38' W, 20 m above sea level) using an augmented design in 2010
100 and 2011. This experimental design is suitable for screening large numbers of new and
101 untried treatments (Federer, 2002). Two hundred and forty un-replicated inbreds were
102 randomly assigned to ten blocks, while six inbred checks (A509, CO125, EP42, EP77,
103 EP80, and PB130) were replicated, being assigned at random to plots within each of the
104 ten blocks; therefore, each block comprised 24 un-replicated inbreds to be screened plus
105 the six inbred checks. The checks were chosen on the basis of their differential
106 performance under inoculation with *F. verticillioides* shown in preliminary evaluations:
107 resistant A509 and EP42; intermediate EP77; susceptible CO125 and PB130
108 (unpublished data). Genotypes evaluated were organized with respect to use (field corn,
109 popcorn or sweet corn), kernel color (white or yellow), kernel type (dent or flint),
110 heterotic group for the field corn inbreds (European, Reid, Lancaster, Northern Flint,
111 Minnesota No 13, other Corn Belt, and miscellaneous), and endosperm mutation (*waxy*,
112 *opaque*, *su1*, *su1se1*, *sh2* or wild). The heterotic group called 'other Corn Belt' included
113 all germplasm from the USA that was neither Reid, nor Lancaster, nor Northern Flint
114 nor Minnesota No13. In each trial, estimates of recorded traits for the un-replicated
115 inbreds were adjusted for block differences which were measured by the inbred checks
116 (Petersen, 1985). Each entry was assigned to a row of 11 plants with 0.18 m spacing
117 between plants and 0.8 m between rows.

118 This study was conducted at the southern Atlantic European Coast which has a
119 unique climate with summer temperatures averaging around 23 °C and relatively mild
120 spring, fall, and winter seasons; the region also experiences high rainfall (from 900 to

121 2000 mm a year), particularly in the fall-winter-spring period. In 2010 and 2011, the
122 mean rainfall in the growing season (May to October) was 66 mm and 57 mm,
123 respectively, while the mean temperature was 19 °C in 2010 and 18 °C in 2011. Some
124 differences in the distribution of the rainfall should be noted as there was considerably
125 less rainfall in August 2010 (7.2 mm) than in 2011 (120 mm).

126 The main corn borer in the Mediterranean area, *Sesamia nonagrioides* (Lef.),
127 preferentially damages the stem and the shank of the plant and has been described as an
128 important vector for *F. verticillioides* infection (Velasco et al., 2002; Velasco et al.,
129 2007; Avantaggiato et al., 2003). The relationship between kernel damage by *S.*
130 *nonagrioides* and fumonisin content has been established (Avantaggiato et al., 2003).
131 However, it is important to note that for our study in these particular environmental
132 conditions, kernel damage by borers tended to be low.

133 2.2. Artificial inoculations and fumonisin determinations

134 Since natural infection cannot guarantee sufficient and homogeneous inoculum levels to
135 adequately differentiate genotypic variation in resistance to *F. verticillioides*
136 (Mesterhazy et al., 2012), we inoculated the plants using a kernel inoculation technique.
137 It has been previously shown that with kernel inoculations the probability of identifying
138 hybrids as resistant when they are actually susceptible in environments that favor
139 fumonisin accumulation is low; although kernel inoculation may overcome low levels
140 of natural resistance, such as that provided by tight husks (Kleinschmidt-DeMasters,
141 2009). In each row (genotype), approximately seven to 14 days after silking, all primary
142 ears were inoculated with 2 ml of a spore suspension of *F. verticillioides*. Each
143 genotype was inoculated according to its singular flowering date. This fungal isolate is
144 an aggressive toxigenic isolate adapted to the local environment and previously isolated
145 from a maize ear; the isolate is deposited in the Culture Collection of the Misión

146 Biológica de Galicia (CSIC), Spain. The spore suspension contained 10^6 spores per ml
147 and was injected into the center of the ear using a four-needle vaccinator which
148 perforated the husks and injured three to four kernels (Reid et al., 1996). Ears from each
149 row (genotype) were collected two months after inoculation and were individually rated
150 for Fusarium ear rot using a seven-point scale (1= no visible disease symptoms, 2 =1-3
151 %, 3 = 4-10 %, 4 = 11-25 %, 5 = 26-50 %, 6 = 51-75 %, and 7 = 76-100 % of kernels
152 exhibiting visual symptoms of infection, respectively) devised by Reid and Zhu (Reid
153 and Zhu, 2005). Each genotype was harvested according to its particular inoculation
154 date. After rating, ears were dried at 35 °C for one week, shelled and a representative
155 kernel sample of approximately 200 g was ground and stored at 4 °C until further
156 chemical analyses. Kernels were ground through a 0.75 mm screen in a Pulverisette 14
157 rotor mill (Fritsch GmbH, Oberstein, Germany).

158 Ground samples were sent to the Food Technology Department of the University of
159 Lleida, Spain, for determination of total fumonisin content (fumonisins B₁, B₂, and B₃)
160 using a commercial ELISA kit (R-Biopharm Rhône Ltd, Glasgow, Scotland, UK). This
161 kit is a competitive enzyme immunoassay for quantification of fumonisin residues in
162 corn. The recovery rate of the test was approximately 60% with a mean coefficient of
163 variation of approximately 8%; specificities for B₁, B₂, and B₃ were 100%, approximately
164 40%, and approximately 100%, respectively, and the detection limit was 0.025 g kg⁻¹.
165 Extraction and preparation of samples, as well as test performance, were carried out as
166 described in the kits.

167 2.3. Statistical analysis

168 Individual analyses of variance were performed for each year as described by Petersen
169 (2005) and Scott and Milliken (1993) with genotypes as fixed effects and blocks as
170 random effects. The inbred check PB130 was removed from the data analysis due to its

171 extremely high levels of fumonisins and a large standard error. The two-way analysis
172 of random year effects and fixed genotype effects was performed with the least square
173 mean estimates of treatments obtained for each year ([Federer et al., 2001](#)). Mean
174 comparisons among genotypes were computed using Fisher's protected LSD.

175 In order to get a representation of the fumonisin data across years we performed
176 a standardization using the z-score scaling: variables recalculated as $(X - \text{mean of } X)/s$,
177 where "s" is the standard deviation. As a result, all variables in the data set have equal
178 means (0) and standard deviations (1) but different ranges.

179 Genotypes were grouped according to their kernel color and type, mutation, use
180 and heterotic group. Analyses of variance and mean comparisons were performed for
181 studying genetic variability for fumonisin content and Fusarium ear rot among these
182 groups. Spearman correlations coefficients between Fusarium ear rot and fumonisin
183 accumulation were computed with the adjusted means for each genotype. All statistical
184 analyses were performed with SAS ([SAS, 2008](#)).

185 **3. Results and discussion**

186 The combined analyses of variance across years detected significant differences among
187 genotypes for Fusarium ear rot and fumonisin content (Table 1). The year x genotype
188 interaction was significant for fumonisin content and was due to changes in genotype
189 ranking rather than magnitude changes across years. For this reason, mean comparison
190 among genotypes for fumonisin content was kept separate for each year, but
191 comparisons for ear rot symptoms were combined across years. Since fumonisin
192 quantification requires expensive chemical analyses and this data indicated that these
193 analyses should be performed in several environments due to the large environment x
194 genotype interaction, it would be advantageous if a simpler less costly trait that was
195 genetically correlated to fumonisin content could be used as an indirect selection
196 criterion for increasing resistance to fumonisin accumulation.

197 Adjusted means for block differences of each genotype evaluated in both years
198 plus the genotype characteristics with respect to use, kernel color, kernel type, heterotic
199 group of field corn inbreds, and endosperm mutation are summarized in Table 2. Fifty
200 eight inbreds from the Misión Biológica de Galicia Bank (A188, A509, A556, A619,
201 A619o2, A630, A635, A637, A638, A641, A652, A654, A662, A666, A670, A680,
202 A681, A682, B93, B98, BP1, BPM2, C68, DS69, EP16, EP17, EP28, EP31, EP32,
203 EP39, EP45, EP47, EP55, EP56, EP57, EP65, EP69, EP72, EP73, EP76, EP77, EP78,
204 F557, F575, F7, G, H95, H99, Mo20W, Oh43, PB57, PB97, PB98, V642, W153R,
205 W572, W64Ao2 and Z77016) plus 3 private inbreds (Private 2,3,and 5) exhibited the
206 lowest values for fumonisin content in 2010 and 2011 and the lowest averaged values
207 across years for Fusarium ear rot severity (Table 2, Figure 1). Groups were made
208 according to the least significant difference obtained for each trait; resistant inbreds do
209 not differ significantly from the best. Inbreds had to be included in the resistant group

210 for the three traits (FB10, FB11 and ear rot) in order to be considered as resistant or
211 susceptible. Two of the more resistant inbreds, A188 and A635, have also been
212 evaluated in crosses with FR1064 (a susceptible inbred) along with another 1587
213 inbreds under silk inoculation with *F. verticillioides* and were among the most resistant
214 to fumonisin accumulation and Fusarium ear rot (Clements and White, 2004; Clements
215 et al., 2004). We also confirmed the susceptibility to fumonisin accumulation of the
216 inbred Va35 that was previously evaluated in the southern United States under kernel
217 inoculation with *F. verticillioides* (Henry et al., 2009). These 61 inbreds included
218 genotypes differing for kernel color, use, kernel type and heterotic group. When
219 analyses of variance was performed to check for differences among these characteristics
220 with respect to *Fusarium* resistance, the difference between white and yellow kernels
221 was significant at 0.10 probability level for fumonisin content, differences between
222 dent and flint kernels were significant at 0.10 probability level for fumonisin content
223 and Fusarium ear rot and the interaction of year x use was significant at 0.05 probability
224 level for both fumonisin accumulation and Fusarium ear rot (Table 3a). White corn had
225 ($p < 0.10$) higher levels of fumonisin than yellow corn (Table 4), but it was still possible
226 to find white inbreds, such as A188, EP65, F557, F575, Mo20W and PB98, with
227 comparable resistance to fumonisin accumulation to that of the most resistant yellow
228 inbreds. Similarly, although the sweet corn inbreds evaluated in this study were less
229 resistant to infection by *F. verticillioides* than the field corn inbreds (just for 2011),
230 there were some, such as V642 (parentage Sugar & Gold ♂) and C68 [derived from the
231 population Whipple Yellow (Harris)], which were grouped with the most resistant 61
232 inbreds (Table 3b). Other authors have already observed variability for resistance to
233 infection by *F. verticillioides* among sweet corn genotypes (Headrick and Pataky, 1991;
234 Nankam and Pataky, 1996). In contrast to results by Löffler et al. (2010), the flint

235 inbreds evaluated in our study had significantly ($p < 0.10$) less fumonisin content and
236 Fusarium ear rot than the dent genotypes. These differences between studies could be
237 due to the different genotypes evaluated; Löffler et al. (2009), (2010) used elite
238 breeding materials of the KWS SAAT AG, Einbeck, Germany, while we used mostly
239 public inbreds that have been released by universities or other public institutions and
240 may represent a greater diversity of germplasm. Among the flint inbreds in our study,
241 those related to the corn landraces from southern Europe, such as EP31, EP32, EP39,
242 EP45, EP65, PB57, PB98, F7, F557, and F575, seemed to be the most promising
243 sources of resistance to fumonisin accumulation.

244 Differences among *waxy* and wild versions of the same inbred were not
245 significant for either ear rot severity or fumonisin accumulation; however, *opaque* and
246 wild versions of the same inbred differed significantly at the 0.10 probability level for
247 fumonisin content (Table 4a). Although *waxy* inbreds tended to have higher levels of
248 fumonisin than their wild counterparts, differences were not significant due to the high
249 standard deviation associated with each mean as a consequence of the low number
250 (four) of inbreds evaluated (Table 4b). Blandino and Reyneri (2007) also observed
251 higher concentrations of fumonisins in *waxy* than in wild genotypes. *Waxy* corn contains
252 only amylopectin and no amylose starch molecules unlike wild corn genotypes that
253 contain both. It has been shown that amylopectin induces FB1 production in *F.*
254 *verticillioides* (Bluhm and Woloshuk, 2005). *Opaque* inbreds had significantly ($p <$
255 0.10) less fumonisin accumulation than their corresponding wild versions (Table 4b).
256 This result does not agree with results reported previously in which *o2* mutants were
257 significantly more susceptible to *F. verticillioides* than their wild isolines (Lanzanova et
258 al., 2009). Therefore, the effect of the *o2* mutant on fumonisin accumulation should be

259 studied in more detail as well as the effect of other mutations which affect the protein
260 profile of the corn kernel.

261 The Spearman correlation coefficients between fumonisin content and Fusarium
262 ear rot were moderate and significant ($r = 0.64$, $p < 0.0001$, across years, $r = 0.56$, $p <$
263 0.0001 , 2010 and 2011 respectively) (Table 5) supporting previous results that showed a
264 better correlation between both traits after wound inoculation than under silk
265 inoculation with *F. verticillioides* (Schaafsma et al., 2006). Fusarium ear rot could be
266 useful for screening multiple inbred lines in a single environment because this trait
267 seemed stable across environments (Henry et al., 2009). Selected inbreds exhibiting
268 resistance could then be tested for fumonisin accumulation in different environments.
269 This breeding procedure would work well when applied to white kernel genotypes or
270 genotypes belonging to the miscellaneous heterotic group because Spearman correlation
271 coefficients between fumonisin content and Fusarium ear rot were high in both years in
272 both of these groups; while selection for reduced Fusarium ear rot would not be a good
273 indirect criterion for diminishing fumonisin content in popcorn and sweet corn.

274 In summary, significant differences for resistance to Fusarium ear rot and
275 fumonisin accumulation were found among the genotypes evaluated in this study.
276 Several of the inbreds could be used to develop new populations and inbred lines with
277 improved resistance. If a pedigree breeding program was used, evaluations for
278 Fusarium ear rot in a single environment could be done on early generations as this trait
279 appeared stable across environments. This could be followed by evaluations of the
280 selected advanced progenies for disease severity as well as the more costly and time
281 consuming quantification of kernel fumonisin content in several environments. This
282 study also showed that it is not necessary to transfer resistance from a given kernel
283 color, type or use to another since acceptable levels of resistance were found in all of

284 the different groups we studied. Finally, to take advantage of the adaptation and
285 heterosis of the heterotic patterns 'Reid Dent' x 'European Flint' and 'Lancaster Dent' x
286 'European Flint', we propose to initiate a pedigree breeding program for obtaining field
287 corn inbreds with the following crosses: EP31 x EP39 and F575 x EP65 (European
288 Flint), B93 x Oh43 and A670 x H95 (Lancaster Dent) and A630 x A635 and A654 x
289 A666 (Reid Dent).

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- 1 Table 1. Mean squares (MS) and degrees of freedom (df) of the analysis of variance of 245 corn inbred lines (240 un-replicated inbreds and five inbred
 2 checks) evaluated for *Fusarium* resistance in two years using an augmented design.

	Fumonisin						Fusarium ear rot					
	2010		2011		Across years		2010		2011		Across years	
	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>
Block	9	485	9	4179			9	1.52*	9	1.10*		
Genotype	222	4369**	221	17076**	236	37900**	218	1.48**	217	1.21**	234	1.81**
Year (Y)					1	3181					1	1.32
Y x G					207	22579**					201	0.72
Error	35	557	34	4009	69	2283	35	0.70	34	0.42	69	0.56

- 3 *, ** Significant at 0.05 and 0.01 probability levels, respectively.

1 Table 2. Adjusted means for block differences of each inbred evaluated for *Fusarium* ear rot
2 severity and fumonisin accumulation (FB) in 2010 and 2011 and each inbred characteristic as related
3 to use, kernel color of field corn inbreds, type of kernel of field corn inbreds, endosperm mutation
4 and heterotic group of field corn inbreds. *Sixty one resistant inbreds to fumonisins are highlighted.

Inbred	Color	Use	Type	Mutant	Heterotic group	FB(10) 2010	FB(11) 2011	Fusarium ear rot
K305	White	Field corn	Dent	Wild	Lancaster	49.64	74.1	2.84
33-16	White	Field corn	Dent	Wild	Other Corn Belt	63.58	71.99	3.22
A188 *	White	Field corn	Dent	Wild	Other Corn Belt	-20.85	30.81	2.24
EP10	White	Field corn	Dent	Wild	Other Corn Belt	1677.66	59.92	4.89
EP81	White	Field corn	Dent	Wild	Other Corn Belt	113.22	204.86	4.57
H105W	White	Field corn	Dent	Wild	Reid	50.18	9.6	2.9
H106W	White	Field corn	Dent	Wild	Reid	1407.04	281.42	5.41
K301	White	Field corn	Dent	Wild	Reid	24.48	55.72	3.75
Mo20W *	White	Field corn	Dent	Wild	Unknown	25.49	29.61	2.33
EP64	White	Field corn	Flint	Wild	European	46.51	16.4	2.51
EP65 *	White	Field corn	Flint	Wild	European	6.67	8.69	2.15
EP66	White	Field corn	Flint	Wild	European	24.64	36.09	2.98
EP71	White	Field corn	Flint	Wild	European	18.28	201.63	3.49
F557 *	White	Field corn	Flint	Wild	European	20.05	32.74	2.41
F575	White	Field corn	Flint	Wild	European	7.86	6.89	2.23
PB40	White	Field corn	Flint	Wild	European	59.77	155.79	3.66
PB98 *	White	Field corn	Flint	Wild	European	-0.09	23.37	0.95
FP1	White	Field corn	nd*	Wild	Miscellaneous	42.5	64.03	2.54
EA3076	Yellow	Field corn	Dent	Wild	European	98.52	163.5	3.68
A295	Yellow	Field corn	Dent	Wild	Lancaster	10.12	54.59	2.94
A427	Yellow	Field corn	Dent	Wild	Lancaster	104.5	154.66	5
A619 *	Yellow	Field corn	Dent	Wild	Lancaster	24.57	28.5	2.41
A624	Yellow	Field corn	Dent	Wild	Lancaster	58.08	-19.63	2.71
A670 *	Yellow	Field corn	Dent	Wild	Lancaster	11.87	15.61	2
A671	Yellow	Field corn	Dent	Wild	Lancaster	70.12	41.64	2.21
A677	Yellow	Field corn	Dent	Wild	Lancaster	136.4	59.15	1.73
A682 *	Yellow	Field corn	Dent	Wild	Lancaster	28.14	38.87	2.26
B93 *	Yellow	Field corn	Dent	Wild	Lancaster	8.64	-51.02	1.57
C123	Yellow	Field corn	Dent	Wild	Lancaster	55.31	214.25	3.17
EP2	Yellow	Field corn	Dent	Wild	Lancaster	45.13	198.62	3.09
H95 *	Yellow	Field corn	Dent	Wild	Lancaster	10.77	27.5	2.07
H99 *	Yellow	Field corn	Dent	Wild	Lancaster	-0.98	44.06	0.77
Mo17	Yellow	Field corn	Dent	Wild	Lancaster	128.93	39.46	3.02
Oh43 *	Yellow	Field corn	Dent	Wild	Lancaster	11.18	33.38	1.47
Oh545	Yellow	Field corn	Dent	Wild	Lancaster	36.22	54.95	3.57
Va35	Yellow	Field corn	Dent	Wild	Lancaster	26.6	140.82	2.44

Private 3 *	Yellow	Field corn	Dent	Wild	Lancaster	-22.04	47.14	1.52
Private 4	Yellow	Field corn	Dent	Wild	Lancaster	586.82	196.31	4.84
Private 6	Yellow	Field corn	Dent	Wild	Lancaster	235.4	181	2.9
LH51	Yellow	Field corn	Dent	Wild	Lancaster	32.54	-8.49	2.54
A495	Yellow	Field corn	Dent	Wild	Minnesota n13	2.84	179.76	3.15
A509 *	Yellow	Field corn	Dent	Wild	Minnesota n13	2.94	20.55	1.89
W117	Yellow	Field corn	Dent	Wild	Minnesota n13	60.64	39.46	3.48
W153R *	Yellow	Field corn	Dent	Wild	Minnesota n13	28.15	40.42	1.6
W552	Yellow	Field corn	Dent	Wild	Minnesota n13	0.96	160.7	2.27
EP57*	Yellow	Field corn	Dent	Wild	Minnesota n13	33.87	5.37	1.85
A673	Yellow	Field corn	Dent	Wild	Miscellaneous	34.1	48.77	2.67
EP51	Yellow	Field corn	Dent	Wild	Miscellaneous	555.49	171.35	4.53
EP55 *	Yellow	Field corn	Dent	Wild	Miscellaneous	12.54	16.98	2.06
EP67	Yellow	Field corn	Dent	Wild	Miscellaneous	71.91	191.47	3.85
EP72 *	Yellow	Field corn	Dent	Wild	Miscellaneous	35.66	46.3	1.79
EP74	Yellow	Field corn	Dent	Wild	Miscellaneous	11.51	64.24	2.54
EP75	Yellow	Field corn	Dent	Wild	Miscellaneous	9.39	85.54	2.94
EP76 *	Yellow	Field corn	Dent	Wild	Miscellaneous	29.92	18.72	1.58
EP77 *	Yellow	Field corn	Dent	Wild	Miscellaneous	9.15	18.83	1.62
EP78 *	Yellow	Field corn	Dent	Wild	Miscellaneous	-17.24	51.06	2.38
Private 2 *	Yellow	Field corn	Dent	Wild	Miscellaneous	-19.03	12.4	2.19
A251	Yellow	Field corn	Dent	Wild	Other Corn Belt	32.57	15.67	2.84
A340	Yellow	Field corn	Dent	Wild	Other Corn Belt	14.41	40.83	3.61
A659	Yellow	Field corn	Dent	Wild	Other Corn Belt	102.78	31.57	3.11
A661	Yellow	Field corn	Dent	Wild	Other Corn Belt	5.01	38.09	2.72
A662 *	Yellow	Field corn	Dent	Wild	Other Corn Belt	2.88	42.91	1.75
A73	Yellow	Field corn	Dent	Wild	Other Corn Belt	59.11	19.11	2.76
B87	Yellow	Field corn	Dent	Wild	Other Corn Belt	28.27	16.98	2.55
B97	Yellow	Field corn	Dent	Wild	Other Corn Belt	654.76	54.57	3.52
B98 *	Yellow	Field corn	Dent	Wild	Other Corn Belt	8.79	-1.23	1.56
B9A	Yellow	Field corn	Dent	Wild	Other Corn Belt	0.9	56.31	2.75
CO106	Yellow	Field corn	Dent	Wild	Other Corn Belt	82.61	62.6	3.68
CO125	Yellow	Field corn	Dent	Wild	Other Corn Belt	18.14	153.84	3.09
CO328	Yellow	Field corn	Dent	Wild	Other Corn Belt	46.83	120.98	3.11
EP27	Yellow	Field corn	Dent	Wild	Other Corn Belt	3.99	144.13	3.03
EP28 *	Yellow	Field corn	Dent	Wild	Other Corn Belt	12.45	58.05	1.93
EP29	Yellow	Field corn	Dent	Wild	Other Corn Belt	118.54	121.91	4.05
EP52	Yellow	Field corn	Dent	Wild	Other Corn Belt	26.92	45.81	2.97
EP56 *	Yellow	Field corn	Dent	Wild	Other Corn Belt	9.35	10.71	1.86
G *	Yellow	Field corn	Dent	Wild	Other Corn Belt	44.51	37.6	2.17
W401	Yellow	Field corn	Dent	Wild	Other Corn Belt	2.13	19.55	2.61
A239	Yellow	Field corn	Dent	Wild	Reid	595.3	87.75	4.36
A554	Yellow	Field corn	Dent	Wild	Reid	14.04	47.14	2.67
A556 *	Yellow	Field corn	Dent	Wild	Reid	8.22	54.43	2.39
A630 *	Yellow	Field corn	Dent	Wild	Reid	23.34	-24.68	2.28

A632	Yellow	Field corn	Dent	Wild	Reid	100.07	73.36	2.38
A634	Yellow	Field corn	Dent	Wild	Reid	43.62	123.45	3.77
A635 *	Yellow	Field corn	Dent	Wild	Reid	11.76	15.35	2.33
A637 *	Yellow	Field corn	Dent	Wild	Reid	11.1	-51.01	2.03
A638 *	Yellow	Field corn	Dent	Wild	Reid	5.52	35.2	1.66
A639	Yellow	Field corn	Dent	Wild	Reid	22.61	215.31	3.38
A641 *	Yellow	Field corn	Dent	Wild	Reid	30.04	8.01	2.25
A652 *	Yellow	Field corn	Dent	Wild	Reid	26.32	97.96	2.16
A654 *	Yellow	Field corn	Dent	Wild	Reid	6.82	9.06	2.23
A657	Yellow	Field corn	Dent	Wild	Reid	550.79	163.18	3.12
A664	Yellow	Field corn	Dent	Wild	Reid	23.33	198.24	3.03
A665	Yellow	Field corn	Dent	Wild	Reid	10.57	63.26	2.49
A666 *	Yellow	Field corn	Dent	Wild	Reid	-23.2	27.25	1.96
A672	Yellow	Field corn	Dent	Wild	Reid	67.32	113.5	3.56
A675	Yellow	Field corn	Dent	Wild	Reid	4	16.62	3.52
A680 *	Yellow	Field corn	Dent	Wild	Reid	45.87	35.06	1.97
A681 *	Yellow	Field corn	Dent	Wild	Reid	34.84	55.2	1.68
B14A	Yellow	Field corn	Dent	Wild	Reid	286.53	101.78	2.95
B37	Yellow	Field corn	Dent	Wild	Reid	394.52	57.43	3.93
B73	Yellow	Field corn	Dent	Wild	Reid	492.8	112.88	2.64
B84	Yellow	Field corn	Dent	Wild	Reid	32.43	50.62	3.24
CM105	Yellow	Field corn	Dent	Wild	Reid	98.04	207.63	4.06
CM109	Yellow	Field corn	Dent	Wild	Reid	169.18	71.49	2.32
CM139	Yellow	Field corn	Dent	Wild	Reid	11.56	111.04	2.68
CM169	Yellow	Field corn	Dent	Wild	Reid	8.5	156.29	2.56
CM99	Yellow	Field corn	Dent	Wild	Reid	14.38	53.81	2.72
H84	Yellow	Field corn	Dent	Wild	Reid	66.75	-21.74	2.85
W182E	Yellow	Field corn	Dent	Wild	Reid	61.3	134.71	3.2
W22	Yellow	Field corn	Dent	Wild	Reid	313.56	104.67	3.8
W570	Yellow	Field corn	Dent	Wild	Reid	103.62	49.16	2.38
W572 *	Yellow	Field corn	Dent	Wild	Reid	29.38	116.49	1.93
W64A	Yellow	Field corn	Dent	Wild	Reid	29.72	191.52	2.45
Private 1	Yellow	Field corn	Dent	Wild	Reid	306.17	287.37	3.54
Private 7	Yellow	Field corn	Dent	Wild	Reid	93.22	50.57	2.86
Private 5 *	Yellow	Field corn	Dent	Wild	Reid	32.18	8.78	1.94
B52	Yellow	Field corn	Dent	Wild	Unknown	1520.65	95.72	4.78
GT114	Yellow	Field corn	Dent	Wild	Unknown	217.45	213.44	4.92
MS1334	Yellow	Field corn	Dent	Wild	Unknown	5.73	-2.6	2.58
EA2024	Yellow	Field corn	Flint	Wild	Argentinian Flint	30.91	54.15	2.71
EP31 *	Yellow	Field corn	Flint	Wild	European	6.17	-44.09	2.07
EP32 *	Yellow	Field corn	Flint	Wild	European	24.52	118.59	2.39
EP39 *	Yellow	Field corn	Flint	Wild	European	14.59	-14.83	1.97
EP42	Yellow	Field corn	Flint	Wild	European	40.24	73.13	3.8
EP43	Yellow	Field corn	Flint	Wild	European	229.26	108.3	3.56
EP45 *	Yellow	Field corn	Flint	Wild	European	23.74	73.06	1.98

EP53	Yellow	Field corn	Flint	Wild	European	49.39	110.42	3.28
EP80	Yellow	Field corn	Flint	Wild	European	25.6	86.77	3.18
F2	Yellow	Field corn	Flint	Wild	European	-22.74	42.27	2.89
F7 *	Yellow	Field corn	Flint	Wild	European	-3.15	5.57	1.27
PB97 *	Yellow	Field corn	Flint	Wild	European	15.36	6.57	1.37
Z77016 *	Yellow	Field corn	Flint	Wild	European	3.56	48.86	2.02
Z78007	Yellow	Field corn	Flint	Wild	European	35.94	23.26	2.8
PB57 *	Yellow	Field corn	Flint	Wild	European	-2.74	9.24	1.48
EP86	Yellow	Field corn	Flint	Wild	European	20.58	56.54	3.33
EP17 *	Yellow	Field corn	Flint	Wild	Miscellaneous	-2.62	47.46	1.62
EP18	Yellow	Field corn	Flint	Wild	Miscellaneous	9.09	160.7	2.11
EP19	Yellow	Field corn	Flint	Wild	Miscellaneous	49.64	49.58	3.4
EP68	Yellow	Field corn	Flint	Wild	Miscellaneous	19.93	78.03	2.65
EP69 *	Yellow	Field corn	Flint	Wild	Miscellaneous	8.36	29.48	2.24
EP73 *	Yellow	Field corn	Flint	Wild	Miscellaneous	1.3	33.25	2.04
EP79	Yellow	Field corn	Flint	Wild	Miscellaneous	2.44	351.07	3.66
EP16 *	Yellow	Field corn	Flint	Wild	Northern Flint	2.47	48.61	2.39
EP3	Yellow	Field corn	Flint	Wild	Northern Flint	3.14	-9.62	2.45
EP4	Yellow	Field corn	Flint	Wild	Northern Flint	58.94	-50.79	2.66
EP46	Yellow	Field corn	Flint	Wild	Northern Flint	53.16	59.78	2.5
EP47 *	Yellow	Field corn	Flint	Wild	Northern Flint	18.23	-39.42	2.08
TD16	Yellow	Field corn	nd*	Wild	Unknown	193.92	143.77	4.94
TD25	Yellow	Field corn	nd*	Wild	Unknown	281.78	55.7	4.43
BP1	-----	Popcorn	-----	Wild	-----	10.28	39.42	1.57
BP2	-----	Popcorn	-----	Wild	-----	32.72	122.78	4.43
BP3	-----	Popcorn	-----	Wild	-----	84.13	43.26	2.12
BPM2 *	-----	Popcorn	-----	Wild	-----	34.15	29.35	2.29
DS28	-----	Popcorn	-----	Wild	-----	46.23	187.53	2.85
DS69 *	-----	Popcorn	-----	Wild	-----	17.31	-14.99	2.37
DS91	-----	Popcorn	-----	Wild	-----	93.09	19.9	3.72
C23sh2	-----	Sweet corn	-----	sh2	-----	96.8	352.8	6.04
C40sh2	-----	Sweet corn	-----	sh2	-----	33.65	417	2.7
EP84	-----	Sweet corn	-----	sh2	-----	73.36	-22.04	3.53
EP85	-----	Sweet corn	-----	sh2	-----	56.71	2.11	2.93
IL101t	-----	Sweet corn	-----	sh2	-----	23.93	87.75	4.96
C13	-----	Sweet corn	-----	su1	-----	64.41	164.11	4.2
C6	-----	Sweet corn	-----	su1	-----	0.77	29.95	2.46
C68 *	-----	Sweet corn	-----	su1	-----	-7.94	20.18	2.33
CO108	-----	Sweet corn	-----	su1	-----	22.89	33.97	3.86
EP58	-----	Sweet corn	-----	su1	-----	71.09	65.64	4.16
EP59	-----	Sweet corn	-----	su1	-----	68.12	76.01	3.07
EP60	-----	Sweet corn	-----	su1	-----	130.72	142.38	2.94
EP61	-----	Sweet corn	-----	su1	-----	42.11	73.68	2.82
EP62	-----	Sweet corn	-----	su1	-----	66.16	45.57	2.92
EP82	-----	Sweet corn	-----	su1	-----	440.78	1057.38	5.41

EP83	-----	Sweet corn	-----	<i>su1</i>	-----	24.91	52.51	2.81
H3	-----	Sweet corn	-----	<i>su1</i>	-----	13.73	258.37	3.94
H5	-----	Sweet corn	-----	<i>su1</i>	-----	67.47	92.55	3.9
H6	-----	Sweet corn	-----	<i>su1</i>	-----	65.43	24.76	3.14
H7	-----	Sweet corn	-----	<i>su1</i>	-----	43.69	45.98	2.79
I453	-----	Sweet corn	-----	<i>su1</i>	-----	22.1	-36.37	2.83
I5125	-----	Sweet corn	-----	<i>su1</i>	-----	193.92	92.54	4.54
I5177	-----	Sweet corn	-----	<i>su1</i>	-----	133.77	542.83	4.87
I5492	-----	Sweet corn	-----	<i>su1</i>	-----	-3.13	64.48	2.81
P39	-----	Sweet corn	-----	<i>su1</i>	-----	-2.93	14.49	3.1
T244su	-----	Sweet corn	-----	<i>su1</i>	-----	28.25	89.6	2.702
TA21	-----	Sweet corn	-----	<i>su1</i>	-----	38.61	23.17	4.23
TA22	-----	Sweet corn	-----	<i>su1</i>	-----	25.58	-11.46	4.03
TA25	-----	Sweet corn	-----	<i>su1</i>	-----	8.13	102.65	2.89
TA26	-----	Sweet corn	-----	<i>su1</i>	-----	2.6	281.22	1.92
TA29	-----	Sweet corn	-----	<i>su1</i>	-----	4.26	88.13	5.06
TA30	-----	Sweet corn	-----	<i>su1</i>	-----	54.21	92.4	4.65
TD101	-----	Sweet corn	-----	<i>su1</i>	-----	9.26	59.04	3.59
V576	-----	Sweet corn	-----	<i>su1</i>	-----	47.54	59.81	4.92
V642 *	-----	Sweet corn	-----	<i>su1</i>	-----	13.43	-0.94	1.2
V663	-----	Sweet corn	-----	<i>su1</i>	-----	37.68	302.13	3.6
V679	-----	Sweet corn	-----	<i>su1</i>	-----	196.51	69.97	3.98
IL767b	-----	Sweet corn	-----	<i>su1sel1</i>	-----	71.08	59.04	3.87
P51	-----	Sweet corn	-----	<i>su1</i>	-----	35.88	85.59	3.97
A619o2 *	-----	Specialty	-----	<i>opaque</i>	Lancaster	39.06	9.91	2.32
Mo17o2	-----	Specialty	-----	<i>opaque</i>	Lancaster	43.72	16.49	3.14
EP3o2	-----	Specialty	-----	<i>opaque</i>	Northern Flint	13.23	54.11	3.43
EP4o2	-----	Specialty	-----	<i>opaque</i>	Northern Flint	11.91	47.93	2.69
EP5o2	-----	Specialty	-----	<i>opaque</i>	Other Corn Belt	42.18	51.94	3.22
A632o2	-----	Specialty	-----	<i>opaque</i>	Reid	28.03	10.42	2.94
B37o2	-----	Specialty	-----	<i>opaque</i>	Reid	84.13	53.2	2.7
W22o2	-----	Specialty	-----	<i>opaque</i>	Reid	58.72	84.14	2.9
W64Ao2 *	-----	Specialty	-----	<i>opaque</i>	Reid	29.33	3.41	2.42
C103wx	-----	Specialty	-----	<i>waxy</i>	Lancaster	468.4	811.92	5.12
Oh43wx	-----	Specialty	-----	<i>waxy</i>	Lancaster	56.84	-10.47	2.11
A632wx	-----	Specialty	-----	<i>waxy</i>	Reid	160.9	154.86	3.5
B37wx	-----	Specialty	-----	<i>waxy</i>	Reid	55.38	163.5	3.27
LSD for checks (0.05)						21.43	57.54	0.5
LSD for new genotypes (0.05)						71.1	190.84	1.67
LSD check vs new genotypes (0.05)						55.05	147.83	1.3

1 *n.d.= not determined.

1¹ recorded in seven-point scale (1= no visible disease symptoms, 2 =1-3 %, 3 = 4-10 %, 4 = 11-25
2 %, 5 = 26-50 % , 6 = 51-75 % , and 7 = 76-100 % of kernels exhibiting visual symptoms of
3 infection, respectively).

1 Table 3a. Mean squares (MS) and degrees of freedom (df) of the analyses of variance of corn kernel
 2 colors (C: white and yellow), uses (U: field corn, popcorn and sweet corn), kernel types (T: dent
 3 and flint) and heterotic groups (G: European, Reid, Lancaster, Northern Flint, Minnesota No 13,
 4 other Corn Belt, and Miscellaneous) for fumonisin content and Fusarium ear rot severity recorded
 5 in years 2010 and 2011.

	Fusarium				Fusarium				
	Fumonisin		ear rot		Fumonisin		ear rot		
	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	
Year (Y)	1	169921**	1	5.62**	Year (Y)	1	12037	1	0.58
Color (C)	1	94281*	1	2.51	Use (U)	2	13336	2	23.90
Y x C	1	88512	1	1.30	Y x U	2	105760**	2	5.07**
Error	303	32764	300	1.17	Error	399	31518	393	1.22
	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>	
Year (Y)	1	41705	1	4.15 [†]	Year (Y)	1	3213	1	1.05
Type (T)	1	104785*	1	3.85*	Group (G)	6	23797	6	1.04
Y x T	1	11089	1	0.01	Y x G	6	17076	6	0.75
Error	269	34835	266	1.11	Error	276	27976	273	1.12

6 *, **, Significant at 0.10 and 0.05 probability levels, respectively.

1 Table 3b. Least square means \pm standard deviations of groups of corn inbreds representing different
 2 kernel colors (white and yellow), kernel types (dent and flint) and uses (sweet corn, field corn and
 3 popcorn) evaluated in 2010 and 2011 for fumonisin content and Fusarium ear rot severity.

		Fumonisin	Fusarium ear rot
Year		mg/kg	(1-7)¹
<i>Color</i>			
White	Across	133 \pm 29 a ²	-----
Yellow	years	81 \pm 11 a	-----
<i>Type</i>			
Dent	Across	96 \pm 13 a ²	2.8 \pm 0.1 a ²
Flint	years	51 \pm 22 a	2.5 \pm 0.1 a
<i>Use</i>			
Sweet	2010	58 \pm 28 b	3.4 \pm 0.2 b
Field		103 \pm 14 ab	2.9 \pm 0.1 c
Popcorn		45 \pm 67 ab	2.7 \pm 0.4 bcd
Sweet	2011	139 \pm 27 a	3.9 \pm 0.2 a
Field		71 \pm 14 b	2.6 \pm 0.1 d
Popcorn		61 \pm 67ab	2.9 \pm 0.4 bcd

4 Within each column, means followed by the same letter did not significantly differ at 0.05
 5 probability level.

6 ¹ Recorded in a seven-point scale 1= no visible disease symptoms, 2 =1-3 %, 3 = 4-10 %, 4 = 11-25
 7 %, 5 = 26-50 %, 6 = 51-75 %, and 7 = 76-100 % of kernels exhibiting visual symptoms of infection,
 8 respectively.

9 ² Within this group, means significantly differed at 0.10 probability level.

1 Table 4a. Mean squares (MS) and degrees of freedom (df) of the analyses of variance of *waxy* vs
 2 wild versions of the same inbreds and *opaque* vs wild versions of the same inbreds evaluated for
 3 fumonisin content and Fusarium ear rot severity in years 2010 and 2011.

	Fusarium			
	Fumonisin		ear rot	
<i>waxy</i>	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>
Year (Y)	1	2770	1	0.24
Mutant (M)	1	26036	1	1.24
Y x M	1	16665	1	0.07
Error	11	59803	11	2.21

<i>opaque</i>				
	<i>df</i>	<i>MS</i>	<i>df</i>	<i>MS</i>
Year (Y)	1	13099	1	2.78 *
Mutant (M)	1	25326 [†]	1	0.04
Y x M	1	10892	1	0.14
Error	28	6947	28	0.68

4 * Significant at 0.10 probability level.

5

1 Table 4b. Least square means \pm standard deviations of corn mutants evaluated in 2010 and 2011 for
2 fumonisin content and Fusarium ear rot severity.

Inbred version	Fumonisin mg/kg	Fusarium ear rot (1-7)¹
<i>waxy</i>	233 \pm 86 a	3.5 \pm 0.5 a
Wild	149 \pm 93 a	2.9 \pm 0.6 a
<i>opaque</i>	37 \pm 21 b	2.8 \pm 0.2 a
Wild	93 \pm 21 a	2.9 \pm 0.2 a

3 Within each column, means followed by the same letter did not significantly differ at 0.10
4 probability level.

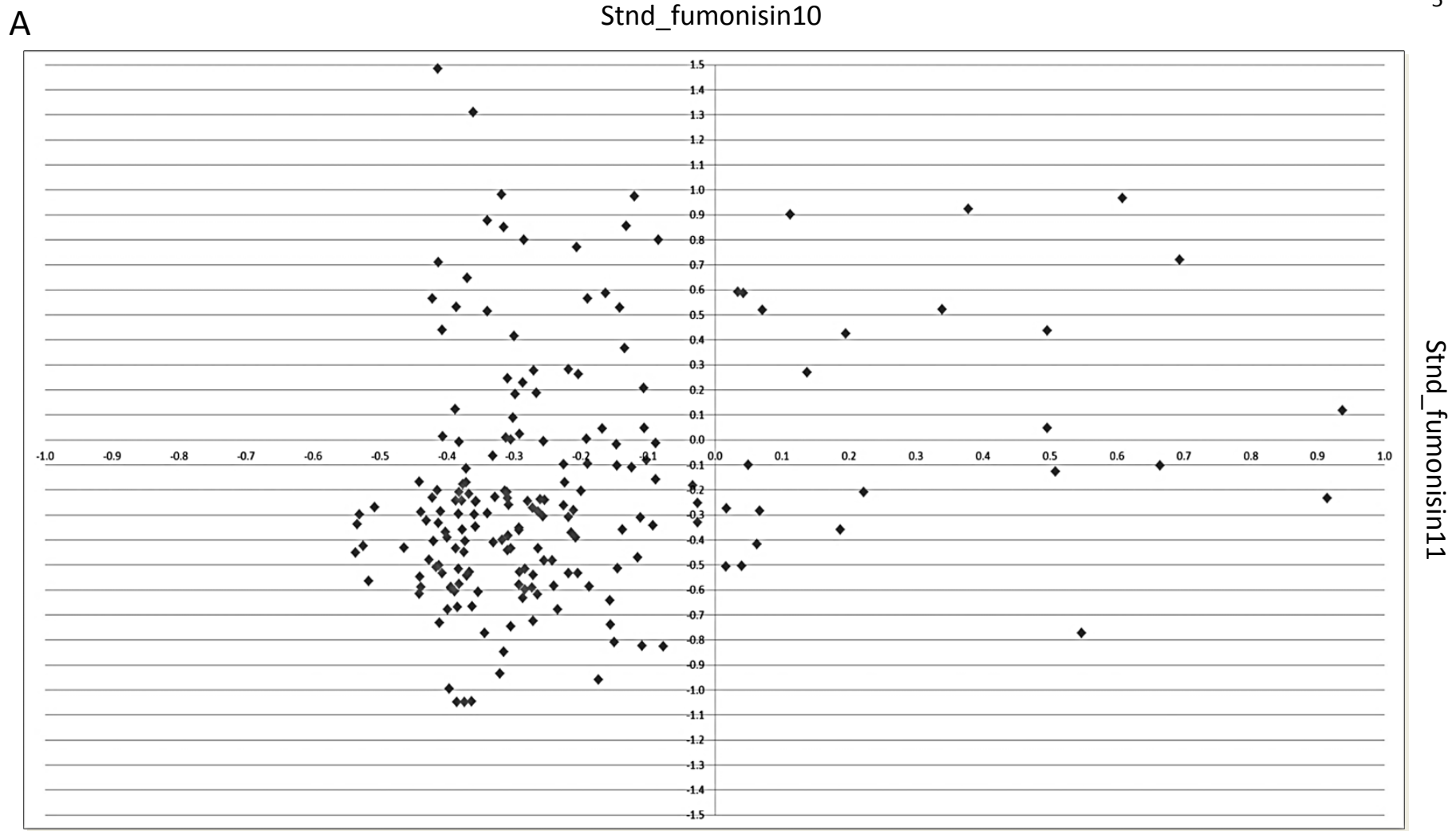
5 ¹ Recorded in a seven-point scale 1= no visible disease symptoms, 2 =1-3 %, 3 = 4-10 %, 4 = 11-25
6 %, 5 = 26-50 %, 6 = 51-75 %, and 7 = 76-100 % of kernels exhibiting visual symptoms of infection,
7 respectively.

1 Table 5. Spearman correlation coefficients (r) between *Fusarium* ear rot severity and fumonisin content in corn inbreds evaluated in two years under
 2 inoculation with *Fusarium verticillioides* and belonging to different groups with respect to kernel color (white and yellow) and type (dent and flint),
 3 use (field corn, popcorn and sweet corn) and heterotic group (European, Reid, Lancaster, Other Corn Belt, and Miscellaneous). The number of inbreds
 4 (N) used for computing each correlation coefficient is indicated.

	All inbreds	White kernel	Yellow kernel	Field corn	Popcorn	Sweet corn	Dent kernel	Flint kernel	Other Belt	European	Lancaster	Reid	Miscellaneous
2010													
r	0.56**	0.73**	0.62**	0.62**	0.70	0.46**	0.63**	0.72**	0.64**	0.73**	0.61**	0.61**	0.79**
N	219	19	132	151	7	39	99	35	23	27	23	44	17
2011													
r	0.56**	0.78**	0.63**	0.65**	0.67	0.50**	0.67**	0.49**	0.56**	0.58**	0.79**	0.59**	0.76**
N	219	19	131	150	7	41	100	34	24	25	25	43	16

5 *, ** Significant at 0.05 and 0.01 probability levels, respectively.

- 1 Figure 1. Standardized distributions of the fumonisin contamination in 2010 and 2011. A) Representation of the whole array of inbred lines evaluated.
- 2 B) Enlarged area representing the genotypes resistant and stable in both years (limited to the genotypes with negative standardized values in both years and included in the resistant group). Kernel color: white (in blue) and yellow (in red); kernel type: dent (solid fill) and flint (unfilled); special corn in green. Heterotic group and type of special corn in the figure B legend.
- 3
- 4



B

