Mycotoxins in maize grains grown in organic and conventional agriculture

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Abstract

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

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

Introduction

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

Mycotoxins are produced by several species of fungi, being *Fusarium* the most common genus in the European Atlantic coast; in southern regions, the most frequent fungus found in maize grains is *F. verticilliodes* Saccardo (=*F. moniliforme* Sheldon) Nirenberg that produces fumonisins (Logrieco et al., 2003), while in colder regions, *F. graminearum* Schwabe that produces deoxynivalenol (DON) could be predominant (Hooker and Schaafsma, 2005). DON is also produced by *F. culmorum* and *F. cereals* (Marin et al., 2013). The Rapid Alert System for Food and Feed of the European Union has registered 14 alerts of fumonisins and 24 of DON between 2008 and 2012 in cereals and bakery products; most of these alerts were for maize samples (RASSF, 2006). Breakfast cereals and baby foods also contained DON, although at lower levels than unprocessed maize kernels (Marin et al., 2013). Fumonisins cause diverse health problems in animals and, in humans, fumonisins could be related with increased incidence of esophageal cancer and neural tube defects and are considered as probably carcinogenic (Bennett and Klich, 2003; IARC, 1993). There are no data supporting the possible mutagenic or carcinogenic effects of DON. IARC has included DON in Group 3 and fumonisins in group 2.4. Although DON is not as toxic as other mycotoxins, it is one of the most common contaminants of cereals worldwide. Acute effects of food poisoning in humans are abdominal pains, dizziness, headache, throat irritation, nausea, vomiting, diarrhea, and bloody stools.

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

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

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

Several authors have searched variability for resistance to fumonisin contamination among maize inbreds or hybrids. For example, Clements et al. (2004) evaluated a collection of inbred lines crossed to a tester under artificial infestation with *Fusarium verticillioides* and found significant differences for fumonisin contamination. Similarly, Afolabi et al. (2007) evaluated a collection of maize inbred lines per se under natural infestation and found differences among maize inbreds and genotype × environment interaction for fumonisin contamination as well as positive correlation between fungi infection and fumonisin contamination only in one of the two Nigerian localities used. Presello et al. (2007) evaluated a group of maize hybrids and found positive correlations between symptom severity and concentration of fumonisins, indicating that genotypic effects for concentration of fumonisins in grain mainly depended on genotypic effects for disease resistance. Löffler et al. (2010) analyzed correlations between mycotoxin concentrations and ear rot rating of 50 inbred lines under artificial infestation and found that the early maturity group flint lines were more susceptible and there were broad ranges and significant genotypic variances as well as genotype x environment interaction variances, but also high heritabilities for ear rot and mycotoxin concentrations. Henry et al. (2009) evaluated a selected group of inbred lines inoculated with either *A. flavus* or *F. verticillioides* and found significant variability for resistance to aflatoxin and fumonisin contamination among maize inbred lines and inbreds resistant to aflatoxin were also resistant to fumonisin contamination. Santiago et al. (2013) evaluated 240 maize inbred lines under kernel inoculation with *Fusarium verticillioides* and found differences for resistance to fumonisin contamination across environments. Bolduan et al. (2009) evaluated in Germany a collection of maize inbreds for mycotoxin contamination, including DON, and found significant genotypic and genotype × environment interaction variances, moderate heritabilities, and high correlations between disease severity and mycotoxin concentrations. DON has been found in naturally infected ears across nine locations in Germany (Magg et al., 2002). Relatively high contaminations of DON were reported in maize genotypes by Hart et al. (1984).

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

Materials and Methods

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

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

Ears from each plot were collected when grains were dry. A representative kernel sample of approximately 200 g was ground and the resulting flour sample was maintained at 4 ºC until performing chemical analyses. Kernels were ground through a 0.75 mm screen in a Pulverisette 14 rotor mill (Fritsch GmbH, Oberstein, Germany). We have performed three replicates of each sample. Mycotoxins were determined using the commercial kit Veratox (Neogen Corp., Lansing MI) a competitive direct ELISA (CD-ELISA) that provides a quantitative analysis of fumonisins and deoxynivalenol (DON) with a lower limit of detection of 0.1 ppm. The kit has the ISO 9001 certificate and the controls provided were 0, 1, 2 , 4 and 6 ppm for fumonisine and 0, 0.5, 1, 2 and 6 ppm for DON. The recovery rate was over 90% in all samples (Neogen Corp. Lansing MI).

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

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

Results and discussion

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

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

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

In the combined analyses of variance, the varieties with higher fumonisin content were Rebordanes(P)C2 (white grain), Martikoenea (yellow grain), and the black-kernel varieties Meiro(P)C2 and Carballeira with white endosperm (Table 1b). The fumonisin content of the tow black-kernel varieties was not significantly different from the yellow varieties with less fumonisin content. The varieties with fumonisin contamination significantly below Rebordanes(P)C2 were all yellow. Santiago et al. (2013) also found that white-grain maize had higher fumonisin content than yellow-grain (P=0.06), but many white inbreds were among those with less contamination. It should be also noted that the varieties with larger growth cycle (Rebordanes(P)C2 and Meiro(P)C2) were among those with higher fumonisin contamination, while the earliest variety Sarreaus(P)C2 was among those with lowest fumonisin contamination. These results are consistent with the fumonisin contamination detected at various stages of development by Cao et al. (2013). Contrarily, Löffler et al. (2010) found that the earliest genotypes were more susceptible than the latest ones.

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

Even though fumonisin was higher than DON contamination, fumonisin content was zero in 25% of the cases while DON was zero only in 3% of the cases (Table 3). Furthermore, the range of fumonisin content was larger than that of DON as in each location at least one variety had 0 mg/kg of fumonisin and the most contaminated variety had from 0.46 to 4.07 mg/kg. These results were obtained under natural contamination, but, under artificial infestation, *F. verticillioides* attack was consistent across environments (Santiago et al. 2013). All varieties had appreciable levels of DON, except Meiro(P)C2 and Rebordanes(P)C2 in Pontevedra 2010. Therefore, attention should be paid to mycotoxin contamination when specific varieties are grown in some locations and years because mycotoxins are almost always present and the amount could rise above the safety levels in some years and locations.

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

a) Means by locations

Locations	Fumonisins			DON		
	Combinado	2010	2011	Combinado	2010	2011
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[a]			0.17[a]	

[a] LSD calculated for comparisons among environments considering each environment as the combination of one year and one location

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

Combined		Conventional		Organic	
Rebordanes(P)C2	1.87 a	Martikoenea	2.40 a	Rebordanes(P)C2	1.25
Martikoenea	1.74 ab	Rebordanes(P)C2	2.33 a	Carballeira	0.98
Meiro(P)C2	1.04 abc	Meiro(P)C2	1.54 ab	Txalín	0.96
Carballeira	1.00 abc	Carballeira	1.02 abc	Martikoenea	0.85
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	Sarreaus(P)C2	0.18 bc	DonostiaC1	0.12
SarreausP)C2	0.13 c	Txalín	0.12 c	Osoro	0.08
DonostiaC1	0.11 c	DonostiaC1	0.11 c	Sarreaus(P)C2	0.08
LSD (0.05)	0.94		1.39		
Media	0.83		1.00		0.61

Table 2. Fumonisins content (ppm) in maize grain of nine open-pollinated populations evaluated in seven environments [a].

Environments		1		2		3		4		5		6		7	
	Rep	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev
Txalín	1	3.211	0.002	0.000	0.000	0.102	0.008	0.000	0.000	0.341	0.011	0.000	0.000	0.000	0.000
Sarreaus(P)C2	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.398	0.017	0.000	0.000
Meiro(P)C2	1	0.000	0.000	2.623	0.005	3.915	0.009	0.000	0.000	0.608	0.018	0.366	0.016	0.491	0.019
DonostiaC1	1	0.000	0.000	0.000	0.000	1.166	0.009	0.000	0.000	0.000	0.000	0.436	0.016	0.000	0.000
Tuy(S)C3	1	0.000	0.000	0.000	0.000	0.911	0.008	0.000	0.000	0.373	0.012	0.385	0.015	0.000	0.000
Osoro	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.451	0.016	3.168	0.015
Rebordanes(P)C2	1	0.709	0.003	0.000	0.000	1.250	0.011	3.669	0.067	0.646	0.011	0.385	0.021	1.875	0.013
Carballeira	1	0.000	0.000	4.544	0.006	6.667	0.008	0.000	0.000	0.629	0.015	0.967	0.016	0.000	0.000
Martikoenea	1	0.000	0.000	8.528	0.008	8.721	0.008	0.000	0.000	0.000	0.000	0.273	0.012	0.000	0.000
Txalín	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.352	0.014	0.000	0.000	0.000	0.000
Sarreaus(P)C2	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.526	0.014	0.230	0.017	1.581	0.013
Meiro(P)C2	2	0.287	0.002	4.250	0.010	4.638	0.003	0.000	0.000	0.545	0.016	0.366	0.021	0.000	0.000
DonostiaC1	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.304	0.013	0.000	0.000
Tuy(S)C3	2	0.894	0.003	0.743	0.006	3.481	0.010	0.000	0.000	0.238	0.020	0.364	0.014	0.000	0.000

Osoro	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.459	0.017	0.000	0.000	0.000	0.000
Rebordanes(P)C2	2	6.012	0.004	7.467	0.008	7.666	0.009	0.000	0.000	0.000	0.000	0.408	0.015	0.773	0.012
Carballeira	2	5.178	0.009	0.000	0.000	0.342	0.008	0.000	0.000	0.000	0.000	0.418	0.019	0.000	0.000
Martikoenea	2	7.200	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6.826	0.013
Txalín	3	5.414	0.006	0.000	0.000	0.014	0.002	0.000	0.000	0.646	0.017	0.000	0.000	0.000	0.000
Sarreaus(P)C2	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.013	0.000	0.000
Meiro(P)C2	3	1.762	0.005	0.320	0.005	0.923	0.006	0.331	0.013	0.117	0.015	0.359	0.017	0.000	0.000
DonostiaC1	3	0.000	0.000	0.114	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.345	0.017	0.000	0.000
Tuy(S)C3	3	5.538	0.003	1.851	0.008	1.862	0.009	0.000	0.000	0.000	0.000	0.157	0.014	0.000	0.000
Osoro	3	0.000	0.000	0.045	0.005	0.828	0.010	0.000	0.000	0.000	0.000	0.303	0.019	1.603	0.012
Rebordanes(P)C2	3	0.000	0.000	3.627	0.003	3.283	0.008	0.000	0.000	0.000	0.000	0.079	0.022	1.396	0.019
Carballeira	3	2.247	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Martikoenea	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.090	0.011	0.189	0.013	4.649	0.016

[a] 1=Heredia 2010, 2=Arkaute 2010, 3=Arkaute 2011, 4=Lobeira 2010, 5=Pontevedra 2010, 6=Lobeira 2011, 7=Pontevedra 2011

Table 3. Deoxynivalenol (DON) content (ppm) in maize grain of nine open-pollinated populations evaluated in seven environments[a].

Environments	1			2		3		4		5		6		7	
	Rep	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev
Txalín	1	1.210	0.008	0.500	0.013	0.240	0.013	0.020	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Sarreaus(P)C2	1	0.940	0.009	1.190	0.013	0.980	0.013	1.310	0.013	0.000	0.000	0.159	0.010	0.000	0.000
Meiro(P)C2	1	1.250	0.012	0.880	0.013	0.600	0.009	0.840	0.013	0.000	0.000	0.166	0.014	1.110	0.108
DonostiaC1	1	0.800	0.013	0.540	0.011	0.262	0.012	0.680	0.011	0.000	0.000	0.110	0.009	0.000	0.000
Tuy(S)C3	1	0.830	0.009	1.440	0.007	1.130	0.013	1.070	0.012	0.000	0.000	0.100	0.005	1.263	0.012
Osoro	1	0.110	0.005	0.970	0.013	0.650	0.011	0.570	0.015	0.080	0.012	0.140	0.010	1.470	0.006
Rebordanes(P)C2	1	1.040	0.014	0.770	0.080	0.410	0.015	0.650	0.014	0.000	0.000	0.160	0.009	0.957	0.008
Carballeira	1	0.090	0.006	0.760	0.008	0.400	0.011	0.000	0.000	0.036	0.010	0.140	0.012	0.406	0.006
Martikoenea	1	0.300	0.006	0.690	0.009	0.340	0.012	0.000	0.000	0.020	0.005	0.220	0.013	0.592	0.009

Txalín	20	0.070	0.008	0.630	0.010	0.310	0.013	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000
Sarreaus(P)C2	20	0.660	0.008	0.620	0.009	0.300	0.013	0.330	0.013	0.554	0.005	0.150	0.013	0.499	0.001
Meiro(P)C2	20	0.460	0.005	0.640	0.010	0.310	0.018	0.510	0.013	0.000	0.012	0.230	0.020	0.827	0.003
DonostiaC1	20	0.220	0.009	0.480	0.009	0.200	0.010	0.000	0.000	0.035	0.010	0.350	0.013	0.488	0.008
Tuy(S)C3	20	0.900	0.044	0.710	0.015	0.480	0.009	0.750	0.014	0.000	0.000	0.000	0.000	0.000	0.000
Osoro	20	0.110	0.009	0.530	0.009	0.260	0.013	0.990	0.009	0.000	0.000	0.150	0.008	0.000	0.000
Rebordanes(P)C2	20	0.000	0.000	0.810	0.013	0.510	0.018	0.900	0.027	0.000	0.000	0.350	0.007	0.986	0.011
Carballeira	20	0.860	0.013	0.730	0.008	0.490	0.010	0.530	0.014	0.000	0.000	0.184	0.010	0.000	0.000
Martikoenea	20	0.610	0.009	0.600	0.014	0.300	0.012	1.000	0.015	0.010	0.004	0.290	0.009	0.550	0.009
Txalín	30	0.130	0.006	1.070	0.005	0.710	0.022	1.250	0.017	0.002	0.002	0.000	0.000	0.080	0.004
Sarreaus(P)C2	30	0.000	0.000	0.820	0.016	0.510	0.021	0.650	0.014	0.035	0.006	0.240	0.014	0.550	0.005

Meiro(P)C2	3	0.110	0.004	0.630	0.009	0.330	0.011	0.940	0.015	0.000	0.000	0.000	0.000	0.622	0.021
DonostiaC1	3	0.130	0.007	0.690	0.008	0.400	0.015	1.000	0.009	0.010	0.002	0.230	0.016	0.270	0.014
Tuy(S)C3	3	0.390	0.009	0.690	0.013	0.340	0.010	0.039	0.016	0.025	0.006	0.000	0.000	0.392	0.007
Osoro	3	0.000	0.000	0.590	0.014	0.230	0.018	0.800	0.015	0.000	0.000	0.230	0.015	1.980	0.016
Rebordanes(P)C2	3	0.280	0.014	0.710	0.037	0.350	0.011	0.040	0.016	0.000	0.000	0.250	0.009	1.800	0.017
Carballeira	3	0.590	0.026	0.830	0.013	0.560	0.014	0.350	0.015	0.000	0.000	0.000	0.000	1.180	0.018
Martikoenea	3	0.140	0.008	0.770	0.014	0.460	0.012	0.410	0.013	0.000	0.000	0.000	0.000	1.220	0.013

[a] 1=Heredia 2010, 2=Arkaute 2010, 3=Arkaute 2011, 4=Lobeira 2010, 5=Pontevedra 2010, 6=Lobeira 2011, 7=Pontevedra 2011