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Fusarium species and chemotypes associated with fusarium head blight and fusarium root rot on wheat in Sardinia

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Environmental conditions in Sardinia (Tyrrhenian Islands) are conducive to fusarium root rot (FRR) and fusarium head blight (FHB). A monitoring survey on wheat was carried out from 2001 to 2013, investigating relations among these diseases and their causal agents. FHB was more frequently encountered in the most recent years while FRR was constantly present throughout the monitored period. By assessing the population composition of the causal agents as well as their genetic chemotypes and *EF-1α* polymorphisms, the study examined whether the two diseases could be differentially associated to a species or a population. *Fusarium culmorum* chemotypes caused both diseases and were detected at different abundances (88% 3-ADON, 12% NIV). *Fusarium graminearum* (15-ADON genetic chemotype) appeared only recently (2013) and in few areas as the causal agent of FHB. In *F. culmorum*, two haplotypes were identified based on an SNP mutation located 34 bp after the first exon of the *EF-1α* partial sequence (60% adenine, 40% thymine); the two populations did not segregate with the chemotype but the A-haplotype was significantly associated with FRR in the Sardinian data set ($P = 0{\cdot}001$), suggesting a possible fitness advantage of the A-haplotype in the establishment of FRR that was neither dependent on the sampling location nor the sampling year. The SNP determining the Sardinian haplotype is distributed worldwide. The question whether the A-haplotype segregates with characters facilitating FRR establishment will require further validation on a specifically sampled international data set.

Keywords: chemotype, *EF-1α* haplotype, *Fusarium cortaderiae*, *Fusarium culmorum*, *Fusarium graminearum*

Introduction

Fusarium foot and root rot (FRR) and fusarium head blight (FHB) represent major devastating diseases of wheat worldwide, including the Sardinian Island. Sardinia is a Tyrrhenian Island located in the western Mediterranean Sea, with an area of 24 089 km^2. Currently durum wheat is cultivated as the principal crop, on more than 37 000 ha (B. Satta, Agenzia Laore Sardegna, Italy). Cultivation techniques rarely consider rotation with other crops or the use of fungicide-coated seeds. These factors contribute to increase the disease potential present in the soil. As a consequence, FRR and FHB may become epidemics. Primary factors include environmental conditions, previously cultivated crop and residue management.

The symptoms of FRR depend essentially on the time of infection: early stage infections lead to pre- or post-emergence mortality of the young seedlings accompanied by browning of the coleoptiles; later stage infections cause brown spots located in the first two or three internodes. In the case of a severe attack, blighted heads (shrivelled or no grains) are scattered in the field, easily observed when the wheat is still immature. Damage caused by FRR may cause up to 50% production loss (Hollaway *et al.*, 2013).

Wheat plants affected by FHB show a partial or a total blighting of the head along with browning of the peduncle, depending on disease severity. Sometimes, during humid environmental conditions, orange-pink sporodochia appear on the spikelet, representing the agamic sporulation stage of the fungus. One of the most important impacts of FHB, together with direct production losses, is the contamination of the kernels with mycotoxins (Scheider *et al.*, 2009). Grain with high mycotoxin levels cannot be used for food and feed products. It has been reported that the toxins are also transported from the roots to the upper plant, suggesting FRR as a contributor to toxin contamination (Covarelli *et al.*, 2012). The European Community has defined strict limits for several mycotoxins regarding the commercialization of unprocessed kernels and the food products obtained from different cereals but, depending on the level of toxin contamination, those limits might not sufficiently protect consumers (Gratz *et al.*, 2014).

Depending on agronomic and environmental conditions, the pathogens preferentially cause one of the two diseases.

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Rarely, both FHB and FRR may constitute a significant problem at the same time and conditions (Scherm *et al.*, 2013). The two disease types, despite sharing large weapon sets for infection (Mudge *et al.*, 2006), do adopt at least partly different mechanisms for their establishment (Desmond *et al.*, 2008; Pasquali *et al.*, 2013).

Fusarium graminearum and *Fusarium culmorum* are, together with *Fusarium pseudograminearum*, the most important causal agents of FHB and FRR worldwide (Goswami & Kistler, 2004; Miedaner *et al.*, 2008; Scherm *et al.*, 2013), the latter mainly found in the southern hemisphere (Obanor & Chakraborty, 2014). Each strain of *F. graminearum* and *F. culmorum* can produce several type-B trichothecenes: *F. graminearum* produces mainly deoxynivalenol (DON) and its acetylated forms 3- and 15-acetyldeoxynivalenol (3- and 15-ADON) or nivalenol (NIV), the three chemotypes known so far (Pasquali & Migheli, 2014); and *F. culmorum* produces DON, 3-ADON or NIV.

This study aimed to: (i) characterize the FHB and FRR populations of Sardinia, including their chemotype-associated genotype; and (ii) test whether it is possible to identify genetic features that are associated with FHB or FRR, based on the hypothesis that the microbe–plant interaction in the two diseases is different and therefore that different populations carrying a diverse set of genetic characters (including toxins) could be favoured in the two pathosystems.

Materials and methods

Sampling

The aetiology of FRR and FHB was monitored from 2008 to 2013 by observing 20 locations in the north, centre and south of Sardinia. Four historical samples were also added from the period 2001–2004. A total of 73 *Fusarium* spp. isolates were collected from plants with symptoms covering all representative areas of durum wheat cultivation in Sardinia (Fig. 1). The samples were collected at the milk stage (GS 77) for both diseases.

Fungal isolations were carried out 1 day after sampling. Basal stem sections (2–3 cm) with browning lesions (FRR) or blighted spikelets (FHB) were surface-sterilized with 2% (v/v) sodium hypochlorite for 2 min and then rinsed three times in sterile distilled water. Each single portion was placed on potato dextrose agar (PDA; Oxoid) and incubated at 25°C under 12 h of alternating light and dark. After 4–6 days, all growing *Fusarium* colonies were removed and transferred to carnation leaf agar (CLA). Single spore cultures were prepared from each colony (Burgess *et al.*, 1994).

Strain characterization and storage

Mycelium from the monosporic cultures grown on CLA was morphologically characterized based on spore size and shape according to Burgess *et al.* (1994). All isolates were stored in 15% (v/v) glycerol at −80°C in the strain collection of the 'Centro per la Conservazione e Valorizzazione della Biodiversità Vegetale', University of Sassari, Italy and in the mycological collection at the Centre de Recherche Public – Gabriel Lippmann, Luxembourg.

Isolates were molecularly characterized as follows. Mycelium was grown for 4 days on V8 medium covered with small pieces of Miracloth (Merck-Millipore) tissue. Colonized Miracloth patches were subsequently collected and transferred to Eppendorf tubes containing 100 μL Tris-EDTA (TE) buffer. Eppendorf tubes were then microwaved for 5 min and 2 μL were used directly for PCR amplification. A partial sequence of the translation elongation factor 1α (*EF-1α*) was amplified in a 50 μL reaction containing 1 × Q5 master mix (New England Biolabs) and primers described in Table 1. PCR conditions are also described in Table 1. Bidirectional sequencing was carried out with a 3500 Genetic Analyzer (Life Technologies). Sequences were verified manually using reference sequences obtained from the Fusarium-ID database as described by O'Donnell *et al.* (2012). Database search procedures were then followed for *Fusarium* species determination using both the *Fusarium* database (http://isolate.fusariumdb.org/blast.php) and the NCBI (National Center for Biotechnology Information) BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome). Single nucleotide polymorphism (SNP) identification in the *EF-1α* gene was carried out using the alignment and assembly options provided by CLC v. 7.0 software (QIAGEN). A comparison with previously available *EF-1α* sequences was performed by downloading all available reference sequences from the nucleotide database in NCBI using keywords '*Fusarium culmorum*' and 'elongation factor' followed by a manual check for the correct sequence correspondence (Table S1). The FHB-derived *F. culmorum* data set from Beyer *et al.* (2014) as well as the data set of Obanor *et al.* (2010), which both provide information on the disease type, were included. The SNP designation was counted for all sequences, comparing the presence of T or A, to distinguish two types of isolates. *Tri12* genotype determination to define the genetic chemotype was performed using the same quick DNA extraction procedure as specified above, using the primers and conditions in Table 1. Positive and negative PCR controls were included in each reaction.

Statistical analysis

To test whether the *EF-1α* polymorphism was independent from the sampling year, the sampling location, the genetic chemotype or the type of disease (FRR versus FHB), chi-square tests with the Monte Carlo option of 10 000 samples and a 99% confidence level were conducted using the statistical software package SPSS v. 19 (IBM) on the Sardinian data set.

Results

Disease incidence of the sampled fields varied during the years and locations, with elevated FHB frequencies in 2009, 2010, 2012 and 2013, while FRR was present throughout the whole period of observation (data not shown).

Seventy-three single-spore isolates were obtained: 44 from durum wheat heads, one from a barley head (PVS-Fu 416), one from *Triticum monococcum* (PVS-Fu 415), one from a durum wheat leaf (PVS-Fu 382), one from a wildtype *Hordeum* head (PVS-Fu 418) and 25 from durum wheat showing FRR symptoms.

Species and chemotype determination

Three species were detected in the survey. *Fusarium culmorum* represented the predominant species (86%) of

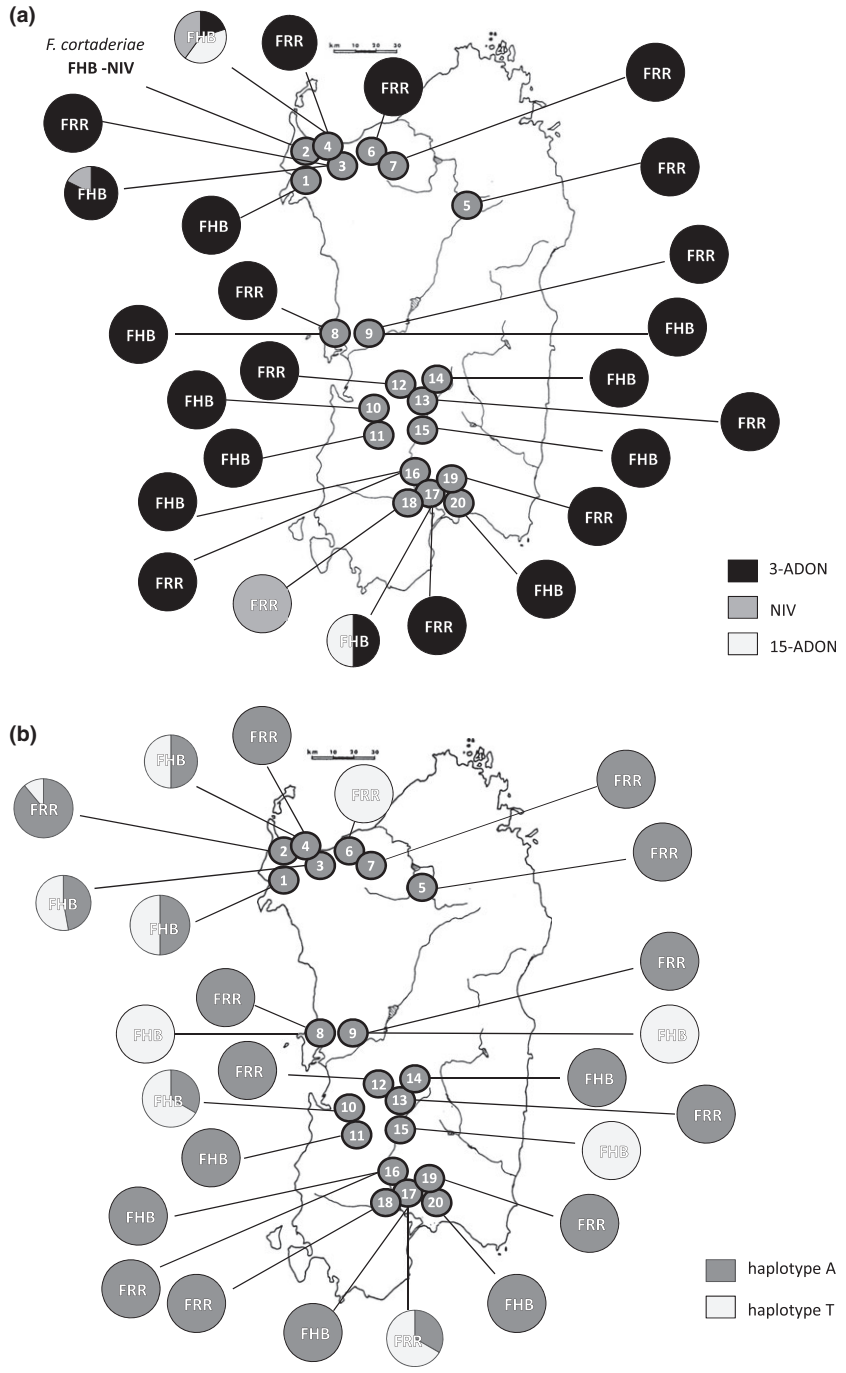

Figure 1 Map of sample sites in Sardinia, Italy (from 2001 to 2013). Percentage of chemotype (a), percentage of haplotype distribution (b). 'FRR' indicates that an isolate was obtained from a plant showing foot and root rot symptoms (isolation from the stem), 'FHB' indicates that an isolate was obtained from a plant showing head blight symptoms (isolation from the head).

the 44 isolates collected from durum wheat heads affected by FHB, while 14% consisted of *F. graminearum* isolates. Finally, one of the isolates, morphologically identified as *F. graminearum*, was re-identified as *F. cortaderiae* upon *EF-1α* sequencing. Among the *F. culmorum* isolates, the 3-ADON chemotype was most prevalent (82%), while the remaining 18% belonged to the NIV chemotype. All *F. graminearum* isolates belonged to the 15-ADON chemotype, while the *F. cortaderiae* isolate was classified as NIV chemotype.

All 25 isolates obtained from the basal stem of durum wheat affected by FRR belonged to *F. culmorum*,

Table 1 Primers and PCR programmes used in this study for *Fusarium* species and chemotype identification

Gene	Primer name	Primer sequence (5′–3′)	Primer amount (μM)	PCR programme	Amplification size (bp)	Chemotype identified[a]
EF-1α	TEF1	ATGGGTAAGGA(A/G) GACAAGAC	0·3	(98°C 2 min) × 1 cycle; (98°C 15 s, 60°C 15 s, 72°C 30 s) × 35 cycles; (72°C 5 min) × 1 cycle	670	–
	TEF2	GGA(G/A)GTACCAGT(G/C)ATCATGTT	0·3			
Tri12	12CON	CATGAGCATGGTGATGTC	0·1	(98°C 2 min) × 1 cycle; (98°C 10 s, 59°C 10 s, 72°C 20 s) × 30 cycles; (72°C 5 min) × 1 cycle		
	12NF	TCTCCTCGTTGTATCTGG	0·1		840	NIV
	12-15F	TACAGCGGTCGCAACTTC	0·1		670	15-ADON
	12-3F	CTTTGGCAAGCCCGTGCA	0·1		410	3-ADON

[a]NIV: nivalenol; 15-ADON: 15-acetyldeoxynivalenol; 3-ADON: 3-acetyldeoxynivalenol.

displaying the 3-ADON genetic chemotype, apart from one isolate (PVS-Fu 398) that was characterized as NIV chemotype. The four *F. culmorum* isolates obtained from durum wheat leaves, from barley, *T. monococcum* and wildtype *Hordeum* heads had 3-ADON chemotype profiles (Table 2; Fig. 1a).

EF-1α diversity and disease association

EF-1α partial sequence analysis of Sardinian *F. culmorum* isolates identified an SNP based on the polymorphic base (T–A) at position 34 after the first exon of the *EF-1α* gene. This SNP defines the two haplotypes in the Sardinian *F. culmorum* population previously identified in an extensive sampling of Sardinian non-cultivated soils (Balmas *et al.*, 2010).

Both haplotypes were distributed homogenously over the Sardinian wheat fields (Fig. 1b), with the A-haplotype being the major component of the Sardinian *F. culmorum* population (60% A-haplotype, 40% T-haplotype; Table 2).

The hypothesis that either the chemotype or the SNP populations are preferentially associated to one of the two types of the disease (FHB or FRR) was examined. A-haplotype isolates, corresponding to haplotype 2 in Balmas *et al.* (2010), were found more frequently associated with FRR ($P = 0{\cdot}001$) than expected under the assumption of independence between haplotype and disease type. Neither location ($P = 0{\cdot}525$) nor chemotype ($P = 0{\cdot}166$) of the isolates had a significant impact on the type of disease. No association between haplotype and chemotype, location or year of sampling could be established.

Screening publicly available data sets on *F. culmorum* *EF-1α* sequences (Table S1) revealed that the A-haplotype form was present in some isolates collected in Tunisia, Syria, Iran (Obanor *et al.*, 2010), Turkey (O'Donnell *et al.*, 2012), Australia (Ward *et al.*, 2008) and Norway (Kristensen *et al.*, 2005) and thus seems to occur worldwide (Fig. S1). No further statistical analysis could be performed to validate the hypothesis of association with FRR due to the very limited information on sample sources: disease type was only described for isolates originating from Tunisia, Iran and Syria, (Obanor *et al.*, 2010), but the small number of isolates did not permit statistical processing with satisfactory power (Table S1).

Discussion

This survey represents the first comprehensive description of *Fusarium*/wheat disease on the island of Sardinia. Sardinian cultural practices and environmental conditions seem to favour the co-occurrence of both FHB and FRR. While *F. pseudograminearum* is the main species associated with FRR diseases in different geographic areas where wheat is cultivated outside Europe (Miedaner *et al.*, 2008), in Sardinia *F. culmorum* appears to cause significant FRR. The Sardinian situation looks very similar to the Turkish (Tunali *et al.*, 2008) and Tunisian (Rebib *et al.*, 2014) ones where *F. culmorum* was the prevalent species associated with crown rot-diseased wheat. Similarly, in Canada *F. culmorum* plays an important role together with *F. pseudograminearum* (Davis *et al.*, 2009) in causing FRR.

In Sardinia FHB on durum wheat is mainly caused by *F. culmorum*. *Fusarium graminearum* is associated exclusively with FHB, but until now as a minor population. This is in contrast with related reports from other regions all over the world, where *F. graminearum* acts as the main causal agent of FHB (Goswami & Kistler, 2004). Similarly, continental Italian regions (Infantino *et al.*, 2012) showed different species abundance, *F. culmorum* being the minor population associated with FHB. The prevalence of *F. culmorum* as the significant FHB agent in Sardinia does not seem to be a temporary event as reported elsewhere (Beyer *et al.*, 2014), but is consistent over the years (2001–2012). *Fusarium culmorum* was frequently reported as a pathogen of colder areas compared to *F. graminearum* (Osborne & Stein, 2007). The results obtained in this survey suggest that this classical distribution scheme is not generally applicable, as *F. culmorum* was identified as the prevalent causal agent of FHB in Sardinia, as in other Mediterranean regions (Kammoun *et al.*, 2010).

In 2013 *F. graminearum* was detected for the first time as an important species causing FHB in Sardinia (over

Table 2 *Fusarium* isolates associated with fusarium foot and root rot (FRR) and fusarium head blight (FHB) in this study

Isolate	Site	Year	Origin[a]	FRR/FHB	Species	Chemotype[b]	*EF-1α* GenBank accession no.	SNP type[c]
PVS-Fu 353	1	2010	P. San Nicola (SS)	FHB	*F. culmorum*	3-ADON	KJ999807	A
PVS-Fu 354	10	2010	Pabillonis (OR)	FHB	*F. culmorum*	3-ADON	KJ999808	A
PVS-Fu 355	16	2010	Benatzu (CA)- A	FHB	*F. culmorum*	3-ADON	KJ999809	A
PVS-Fu 356	16	2010	Benatzu (CA)- B	FHB	*F. culmorum*	3-ADON	KJ999810	A
PVS-Fu 357	10	2010	Pabillonis (OR)	FHB	*F. culmorum*	3-ADON	KJ999811	T
PVS-Fu 358	1	2010	P. San Nicola (SS)- A	FHB	*F. culmorum*	3-ADON	KJ999812	A
PVS-Fu 359	1	2010	P. San Nicola (SS)- B	FHB	*F. culmorum*	3-ADON	KJ999813	T
PVS-Fu 360	9	2010	S. Lucia (OR)	FHB	*F. culmorum*	3-ADON	KJ999814	T
PVS-Fu 361	3	2004	Ottava (SS)	FHB	*F. culmorum*	3-ADON	KJ999815	A
PVS-Fu 362	3	2012	Ottava (SS)	FHB	*F. culmorum*	3-ADON	KJ999816	A
PVS-Fu 363	3	2009	Ottava (SS)- A	FHB	*F. culmorum*	3-ADON	KJ999817	A
PVS-Fu 364	3	2009	Ottava (SS)- A1	FHB	*F. culmorum*	3-ADON	KJ999818	A
PVS-Fu 365	3	2009	Ottava (SS)- B	FHB	*F. culmorum*	3-ADON	KJ999819	A
PVS-Fu 366	3	2009	Ottava (SS)- B1	FHB	*F. culmorum*	3-ADON	KJ999820	A
PVS-Fu 367	3	2009	Ottava (SS)- E	FHB	*F. culmorum*	NIV	KJ999821	T
PVS-Fu 368	3	2009	Ottava (SS)- E1	FHB	*F. culmorum*	NIV	KJ999822	T
PVS-Fu 369	3	2009	Ottava (SS)- H	FHB	*F. culmorum*	3-ADON	KJ999823	T
PVS-Fu 370	3	2009	Ottava (SS)- H1	FHB	*F. culmorum*	3-ADON	KJ999824	T
PVS-Fu 371	3	2009	Ottava (SS)- I	FHB	*F. culmorum*	3-ADON	KJ999825	T
PVS-Fu 372	3	2009	Ottava (SS)- M	FHB	*F. culmorum*	3-ADON	KJ999826	T
PVS-Fu 373	3	2009	Ottava (SS)- P	FHB	*F. culmorum*	NIV	KJ999827	T
PVS-Fu 374	3	2009	Ottava (SS)- S	FHB	*F. culmorum*	3-ADON	KJ999828	T
PVS-Fu 375	3	2009	Ottava (SS)- V	FHB	*F. culmorum*	3-ADON	KJ999829	T
PVS-Fu 376	3	2009	Ottava (SS)- Z	FHB	*F. culmorum*	3-ADON	KJ999830	A
PVS-Fu 377	14	2010	Sarcidano (CA)	FHB	*F. culmorum*	3-ADON	KJ999831	A
PVS-Fu 378	15	2010	Senorbì (CA)	FHB	*F. culmorum*	3-ADON	KJ999832	T
PVS-Fu 379	20	2010	Settimo San Pietro (CA)	FHB	*F. culmorum*	3-ADON	KJ999833	A
PVS-Fu 381	11	2010	San Gavino (OR)	FHB	*F. culmorum*	3-ADON	KJ999834	A
PVS-Fu 382	6	2010	Tergu (SS)	Leaf	*F. culmorum*	3-ADON	KJ999835	T
PVS-Fu 383	16	2010	Benatzu (CA)	FRR	*F. culmorum*	3-ADON	KJ999836	A
PVS-Fu 384	–	2002	–	FRR	*F. culmorum*	3-ADON	KJ999837	A
PVS-Fu 385	4	2009	P. Torres (SS)	FRR	*F. culmorum*	3-ADON	KJ999838	A
PVS-Fu 386	15	2010	Senorbì (CA)	FHB	*F. culmorum*	3-ADON	KJ999839	T
PVS-Fu 387	3	2008	Ottava (SS)	FRR	*F. culmorum*	3-ADON	KJ999840	T
PVS-Fu 388	3	2001	Ottava (SS)	FRR	*F. culmorum*	3-ADON	KJ999841	A
PVS-Fu 389	3	2009	Ottava (SS)- A	FRR	*F. culmorum*	3-ADON	KJ999842	A
PVS-Fu 390	3	2009	Ottava (SS)- B	FRR	*F. culmorum*	3-ADON	KJ999843	A
PVS-Fu 391	3	2009	Ottava (SS)- C	FRR	*F. culmorum*	3-ADON	KJ999844	A
PVS-Fu 392	3	2009	Ottava (SS)- D	FRR	*F. culmorum*	3-ADON	KJ999845	A
PVS-Fu 393	3	2009	Ottava (SS)- E	FRR	*F. culmorum*	3-ADON	KJ999846	A
PVS-Fu 394	3	2009	Ottava (SS)- F	FRR	*F. culmorum*	3-ADON	KJ999847	A
PVS-Fu 395	3	2009	Ottava (SS)- G	FRR	*F. culmorum*	3-ADON	KJ999848	A
PVS-Fu 396	5	2010	Pietrafitta (OT)	FRR	*F. culmorum*	3-ADON	KJ999849	A
PVS-Fu 397	9	2009	S. Lucia (OR)	FRR	*F. culmorum*	3-ADON	KJ999850	A
PVS-Fu 398	18	2010	Serramanna (CA)	FRR	*F. culmorum*	NIV	KJ999851	A
PVS-Fu 399	19	2010	Sinnai (CA)	FRR	*F. culmorum*	3-ADON	KJ999852	A
PVS-Fu 408	13	2010	Suelli (CA)- A	FRR	*F. culmorum*	3-ADON	KJ999853	A
PVS-Fu 409	13	2010	Suelli (CA)- B	FRR	*F. culmorum*	3-ADON	KJ999854	A
PVS-Fu410	17	2001	Ussana (CA)	FRR	*F. culmorum*	3-ADON	KJ999855	T
PVS-Fu 411	17	2010	Ussana (CA)	FRR	*F. culmorum*	3-ADON	KJ999856	A
PVS-Fu 412	12	2010	Villamar (OR)- A	FRR	*F. culmorum*	3-ADON	KJ999857	A
PVS-Fu 413	12	2010	Villamar (OR)- B	FRR	*F. culmorum*	3-ADON	KJ999858	A
PVS-Fu 415	4	2013	P. Torres (SS)	FHB	*F. culmorum*	3-ADON	KJ999859	A
PVS-Fu 416	1	2010	P. S. Nicola (SS)	FHB	*F. culmorum*	3-ADON	KJ999860	T
PVS-Fu 418	10	2010	Pabillonis (OR)	FHB	*F. culmorum*	3-ADON	KJ999861	T
PVS-Fu 419	9	2013	S. Lucia (OR)	FHB	*F. culmorum*	3-ADON	KJ999862	T
PVS-Fu 422	4	2013	P. Torres (SS)- E	FHB	*F. culmorum*	NIV	KJ999863	T
PVS-Fu 423	17	2013	Ussana (CA)	FHB	*F. culmorum*	3-ADON	KJ999864	A

(continued)

Table 2 (continued)

Isolate	Site	Year	Origin[a]	FRR/FHB	Species	Chemotype[b]	*EF-1α* GenBank accession no.	SNP type[c]
PVS-Fu 424	8	2013	Cabras (OR)	FHB	*F. culmorum*	3-ADON	KJ999865	T
PVS-Fu 425	4	2013	P. Torres (SS)- B2	FHB	*F. culmorum*	3-ADON	KJ999866	T
PVS-Fu 426	4	2013	P. Torres (SS)- D1	FHB	*F. culmorum*	NIV	KJ999867	T
PVS-Fu 427	4	2013	P. Torres (SS)- F	FHB	*F. culmorum*	NIV	KJ999868	T
PVS-Fu 428	4	2013	P. Torres (SS)- L1	FHB	*F. culmorum*	NIV	KJ999869	T
PVS-Fu 429	17	2013	Ussana (CA)	FRR	*F. culmorum*	3-ADON	KJ999870	T
PVS-Fu 430	8	2013	Cabras (OR)	FRR	*F. culmorum*	3-ADON	KJ999871	A
PVS-Fu 431	7	2013	Sedini (SS)	FRR	*F. culmorum*	3-ADON	KJ999872	A
PVS-Fu 432	4	2013	P. Torres (SS)- D2	FHB	*F. graminearum*	15-ADON	–	–
PVS-Fu 433	2	2013	Stintino (SS)	FHB	*F. cortaderiae*	NIV	–	–
PVS-Fu 434	17	2013	Ussana (CA)	FHB	*F. graminearum*	15-ADON	–	–
PVS-Fu 435	4	2013	P. Torres (SS)- L2	FHB	*F. graminearum*	15-ADON	–	–
PVS-Fu 436	4	2013	P. Torres (SS)- H1	FHB	*F. graminearum*	15-ADON	–	–
PVS-Fu 437	4	2013	P. Torres (SS)- H2	FHB	*F. graminearum*	15-ADON	–	–
PVS-Fu 906	3	2009	Ottava (SS)- Q	FHB	*F. culmorum*	3-ADON	KJ999873 (A)	A

[a]Multiple isolates from the same plant portion are indicated with the same uppercase letter.
[b]3-acetyldeoxynivalenol, 3-ADON; 15-acetyldeoxynivalenol, 15-ADON; nivalenol, NIV.
[c]Single nucleotide polymorphism (SNP) at nucleotide 34 of the first exon of *EF-1α* (Balmas *et al.*, 2010).

30% of FHB being caused by *F. graminearum*), suggesting that the pathogen had found more favourable conditions during this year or that an emerging population has some competitive advantages. Additional samplings coupled to meteorological and agronomical information are needed to further elucidate the shift in species incidence on the disease.

Interestingly, both *F. graminearum* and *F. culmorum* were isolated (PVS-Fu 426 and 432; PVS-Fu 428 and 435) from two heads collected in 2013 from the same field (Table 2). This confirms the potential role of co-infection as shown in Poland in a recent survey (Kuzdraliński *et al.*, 2014) where *F. graminearum* is the major component of stem disease colonization also able to favour its association with *F. culmorum*. The only *F. cortaderiae* isolate from Sardinia was associated with FHB. Moreover, *F. cortaderiae*, a supposedly southern hemisphere species described by Ward *et al.* (2002), was recently reported in France (Boutigny *et al.*, 2014) suggesting that the species may come across suitable conditions in European climates, too. From the current study it is evident that a geographically isolated area like the island of Sardinia is not immune to the introduction of new species (Migheli *et al.*, 2009) causing further diseases. The introduction of the species into a new environment via seed contamination is a possible hypothesis (Boutigny *et al.*, 2014). This phenomenon has been already observed in wheat (Duthie & Hall, 1987) and is supposed to be the main mechanism of global spread of soilborne *Fusarium* diseases (Garibaldi *et al.*, 2004).

The Sardininan *F. graminearum* population is composed exclusively of 15-ADON isolates. This finding is in line with observations in Italy and in other parts of Europe where 15-ADON is the predominant population (Pasquali & Migheli, 2014). Within the *F. culmorum* isolates, the NIV population is quite infrequent (12%), similar to the situation reported in Germany (Miedaner *et al.*, 2013) or Tunisia (Kammoun *et al.*, 2010). From a food safety perspective, the information on which chemotype is present and prevalent in a certain area may help to establish the level of potential risk of toxin detection. The identification of a small NIV population in Sardinian wheat may suggest a limited impact of NIV in Sardinian crops, but at the same time it requires a constant monitoring effort as a chemotype shift may occur over time (Pasquali & Migheli, 2014).

As reported elsewhere and in the current survey, the presence of more than one chemotype of *F. culmorum* was detected in the same field, perhaps a common finding (Jennings *et al.*, 2004; Pasquali *et al.*, 2010; Boutigny *et al.*, 2014) that suggests that chemotypes may coexist without strongly interfering with each other.

Hypotheses on the prevalence of a certain chemotype (associated with a different toxigenic risk) have been formulated and include the effect of climatic conditions, the use of fungicides, the role of previous crops as well as the mechanisms of interaction with the plant (Pasquali & Migheli, 2014). This current study found that a certain chemotype was not associated with a particular disease type (FRR or FHB), implying the absence of a potential advantage of one chemotype over the other during establishment of FRR or FHB, at least based on these epidemiological data. This confirms that minor toxin type differences do not play a crucial role in disease establishment, but most probably have some specific role in disease progression (Maier *et al.*, 2006).

Fusarium culmorum is a single phylogenetic species that presents a certain level of polymorphism, which suggested the possible existence of sexual recombination (Scherm *et al.*, 2013).

Two haplotypes of *EF-1α* were found within the characterized Sardinian population, confirming results of previous sampling carried out in Sardinian soils (Balmas *et al.*, 2010). Both haplotypes have been sampled in several areas in the same years, supporting the hypothesis that the two haplotypes coexist in Sardinia. Given the significant difference in abundance depending on the disease type, it can be hypothesized that the A-haplotype is somewhat favoured in FRR establishment. The same *EF-1α* sequence is also present in an international data set from other regions and continents where FRR is frequently reported (Tunisia, Iran, Turkey, Australia; Table S1). Unfortunately, only limited information is available in public databases, hence sufficient evidence for the association of the A-haplotype with FRR outside Sardinia is lacking at present. Further analysis looking for phenotypic differences associated with the two *F. culmorum* haplotypes is warranted in order to identify potential characters that may lead to the establishment of a certain disease type on wheat. Importantly, this study showed that disease specificity on wheat can be associated not only with different species (Obanor & Chakraborty, 2014) but also with different populations within a species, in this case *F. culmorum*. As reported in *Fusarium fujikuroi* for fumonisin production, a single SNP in the *EF-1α* sequence may be associated with complex characters, probably associated with a subpopulation within the species (Suga *et al.*, 2014).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Alignment of a representative Sardinian A-haplotype partial *EF-1α* sequence (Ef1_365_SARDINIA_H04) and a representative Sardinian T-haplotype partial *EF-1α* sequence (Ef1_360_SARDINIA_C04) with all *Fusarium culmorum EF-1α* partial sequences present in public databases being identical to the A-haplotype.

Table S1. *Fusarium culmorum EF-1α* gene sequence information used in this study, including NCBI accession numbers, annotations, origin of the isolate and reference publication.