A survey of *Fusarium* species associated with wheat and grass stem bases in northern Turkey

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The *Fusarium* species associated with crown and root rot diseases of wheat have been studied intensively in many parts of the world. However, there are no detailed reports on the relative importance and distribution of *Fusarium* species associated with crown and root rot of wheat in Turkey. Therefore, a survey of *Fusarium* species associated with randomly collected wheat stem bases from arbitrarily selected sites was conducted in three key agroecological wheat production regions in northern Turkey. The *Fusarium* species associated with stem bases of six grass species were also assessed. Fifteen *Fusarium* species were isolated from wheat stem bases. Three of these species, and two additional species were isolated from the grasses. The species isolated included the important crown and foot rot pathogens *F. culmorum* and *F. pseudograminearum,* as well as a number of secondary colonizers and saprobic species. Of the pathogenic species isolated, *F. culmorum* was most prevalent, being isolated from 28% of sites surveyed at an isolation frequency range from individual sites of 2% to 16%, whereas *F. pseudograminearum* was isolated from 8% of sites at an isolation frequency range of 2% to 4%. It seems that, at present, *Fusarium* species do not pose a major threat to wheat production in northern Turkey. The implications of these findings are discussed.

Key words: agroecological region, crown rot, *Fusarium culmorum,* soil-borne diseases.

Members of the genus *Fusarium* are found throughout the world in most bioclimatic regions (Burgess and Summerell 1992), and a number of species are commonly associated with cereals in cropping systems. These species include the important pathogens causing head blight (*F. graminearum* Schwabe), crown rot (*F. pseudograminearum* O'Donnell & T. Aoki) and foot rot (*F. culmorum* [W. G. Smith] Sacc.), as well as the less aggressive pathogen *F. avenaceum* Sacc. Wollenw., and saprobic species such as *F. acuminatum* Ell. & Eve and *F. equiseti* (Corda) Sacc.

Soil-borne diseases are reported to be a recent limitation to Turkish wheat production with studies implicating a number of causal agents including *F. graminearum* and *F. culmorum* as key root and foot rot disease agents (Aktas *et al.* 1997). Within the genus *Fusarium*, five moderately pathogenic or saprobic species were found to be associated with necrotic wheat stem bases collected from randomly selected fields in the Erzurum region of Turkey (Demirci and Dane 2003). These species were *F. acuminatum, F. equiseti, F. oxysporum* Schlecht. Emend. Snyder & Hansen, *F. solani* (H. Mart.) Sacc. and *F. tabacinum sensu lato* (van Beyma) W. Gams (assumed to be *Plectosporium tabacinum* (van Beyma) M.E. Palm, W. Gams & Nirenberg). However, these species were only weakly to moderately pathogenic on wheat in subsequent pathogenicity studies despite being collected from diseased plants (Demirci and Dane 2003).

There is no information on the potential associations of *Fusarium* pathogens of cereals in grassland ecosystems in Turkey, despite reports from other countries that grasses can have a role in the epidemiology of diseases caused by these pathogens (Crofts *et al.* 1988, Burgess *et al.* 2001).

As soil-borne diseases typically build up from low levels initially it is important to understand the range of species associated with cropping even if they have a relatively low incidence or there is limited symptom development under current farming systems. The isolation and identification of *Fusarium* species from diseased as well as asymptomatic tissue is necessary to fully appreciate the underlying range of species associated with the cropping system (Hudec and Rohacik 2003). This information can be subsequently applied to the identification of potentially limiting species that exist within the system and the assessment of the impact of changes in farming systems on diseases caused by these species.

Such strategies have been previously reported for the study of wheat stem rot complexes (Pettitt *et al.* 2003), but no random survey data are available for the *Fusarium* communities associated with wheat stems in Turkey as previous studies have been biased towards collection of visibly diseased plant tissue (Demirci and Dane 2003). Surveys based on the arbitrary selection of sites, and the collection of plants at random provide a scientific basis for evaluating the underlying range and potential of specific microbial communities. These communities have the potential to significantly limit production in subsequent growing seasons. Knowledge of the aetiology of root and crown disease complexes is an essential prerequisite to the implementation of effective control strategies.

Furthermore, the deployment of host cultivars with appropriate disease resistance is difficult unless the underlying spectrum of species within specific microbial communities, and the relative importance of each of these species, is fully understood (Waalwijk *et al.* 2003).

The aim of the current study was to provide quantitative data on the community of *Fusarium* species associated with randomly collected wheat stem bases from arbitrarily selected fields, to determine their relative frequency of isolation and the overall *Fusarium* community structure, and to identify intermediate grass hosts of known *Fusarium* wheat pathogens in three agroecological regions of northern Turkey.

Materials and Methods

Wheat

Samples of physiologically mature winter or spring wheat were collected in June and July 2003 from a total of 25 sites across three broad agroecological regions of northern Turkey (Fig. 1). The regions selected were the west coast of Marmara, the west Black Sea region, and east Central Anatolia, which each have differing average annual temperatures (12.5 °C, 10.9 °C, and 9.1 °C, respectively) and average annual precipitation (980 mm, 340 mm, and 280 mm). Sites were selected arbitrarily based on an approximate separation distance of 25 km. Fifty plants were collected at random from each site in

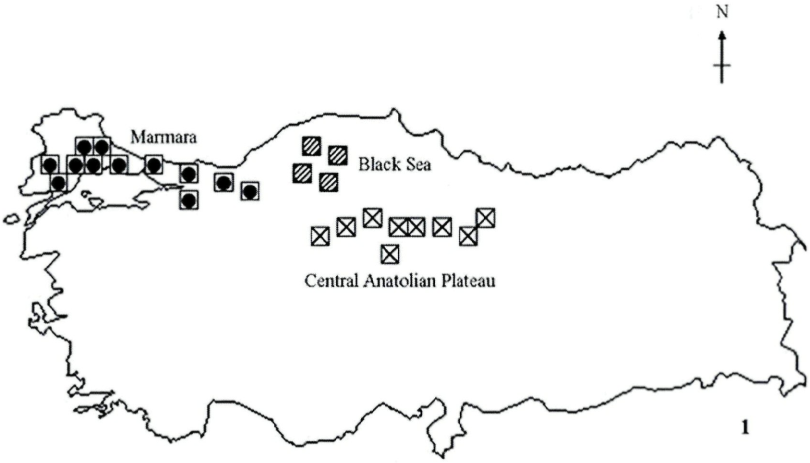

Fig. 1. Sampling sites in the three agroecological regions of northern Turkey; West Coast of Marmara (●), West Black Sea (▨) and East Central Anatolia (☒)

a zigzag pattern beginning 20 m from the edge of the field. Each horizontal line of the zigzag was five meters in length and five plants were collected at the junction of each horizontal line. Plants were transported to the laboratory in paper bags and were washed and plated within a week of collection.

The outer leaves and leaf sheath of the leading tiller of each plant were removed and stems were washed with tap water before being surface-sterilized with 70 % ethanol for 3 min. Single stem sections, approximately 2 – 3 cm in length, including the crown and stem base were removed aseptically from each stem and plated on Peptone-PCNB Agar (PPA), a selective isolation medium for *Fusarium* (Burgess *et al.* 1994). Plates were incubated at 25 °C under alternating light and dark for 7 d, and colonies developing on PPA were subcultured to carnation leaf agar (CLA) (Fisher *et al.* 1982). Isolates were then transferred to CLA and Potato Dextrose Agar (PDA) using single germinated conidia and identified to species level based on appropriate taxonomic criteria (Booth *et al.* 1978, Gerlach and Nirenberg 1982, Nelson *et al.* 1983, Burgess *et al.* 1994, Nirenberg 1995, Leslie and Klein 1996).

Described morphological characters were used to differentiate the majority of isolates in the current study (Booth *et al.* 1978, Gerlach and Nirenberg 1982, Burgess *et al.* 1994). Additional biological and molecular techniques were used when species could not be accurately resolved using morphological characters alone. The utility of a polyphasic approach for the identification of a number of *Fusarium* species has been previously documented (Summerell *et al.* 2003).

The identity of all morphologically identified *F. pseudograminearum* isolates were confirmed by comparison to reference strains of *F. pseudograminearum* from Kansas State University (KSU11445, KSU11446) using Amplified Fragment Length Polymorphism (AFLP) analysis (Zeller *et al.* 2000) with three primer pairs (data not shown).

Isolates putatively identified as *F. proliferatum* (Matsushima) Nirenberg were crossed with the standard testers *G. intermedia* (Peck) Murrill, *MAT-1* (Fungal Genetic Stocks Centre (FGSC) 7615) and *G. intermedia, MAT-2* (FGSC 7614) using standard crossing protocols to confirm identity via sexual compatibility (Leslie and Klein 1996) as this species has not been previously reported from Turkey, and cannot be identified using morphological characters alone. All crosses were repeated once.

The identity of all putative *F. torulosum* (Berk. & Curt.) isolates was confirmed by measuring growth on PDA and chlamydospore production on low nutrient agar (SNA) (Nirenberg 1995). The DNA from 2 putative isolates of *F. torulosum* was extracted using a FastDNA® Kit (Qbiogene, Inc., Irvine, CA, USA.) according to the manufacturer's instructions and the translation elongation factor-1α *(Tef-1)* gene was amplified for further confirmation (Kristensen *et al.* 2005). The forward and reverse complements of the gene were sequenced using an ABI3700 automated sequencer at the Kansas State University sequencing facility. The sequences were aligned using ClustalX (V1.83) (Thompson *et al.* 1997) with sequences obtained from GenBank [species (GenBank accession number/s)]: *F. torulosum* (AJ543607, AJ543612, AJ543614, AJ543535), *F. sambucinum* (AJ543604, AJ543605), *F. venenatum* (AJ543634, AJ543635) and *F. oxysporum* (DQ016289). Phylogenetic analyses were conducted on the aligned sequences using PAUP 4.0b10 (Swofford 1998) with all alignment gaps treated as missing data. A heuristic search with 1000 random replications and using *F. oxysporum* as outgroup resulted in a single maximum parsimony tree, and clade stability was assessed using 1000 bootstrap replicates with random sequence addition.

The *Fusarium* isolation data from all sites within the three broad agroecological regions were analyzed using Primer-E v.5 software. Isolation data were converted into a similarity matrix using the Bray-Curtis coefficient with a square root transformation to downweight highly abundant species (Clarke and Warwick 2001). Non-metric multidimensional scaling was applied to the Bray-Curtis similarity matrix to provide a graphical representation of the ranks of similarities of the sites within the regions studied (Clarke and Warwick 2001). The diversity and range of species isolated from wheat stem bases in each region were further analyzed by calculating Simpson's Index of Diversity, Margalef's Index of species richness and Pielou's evenness index (Clarke and Warwick 2001).

Grasses

Seven grass sites were selected based on the presence of relatively undisturbed stands of annual and perennial grasses, and the presence of an inflorescence to enable species level identification (Tab. 1). Ten plants were selected arbitrarily from each site and the outer leaves and leaf sheath were removed from one stem per plant. Stems were washed and surface-sterilized as for wheat samples. Two 1-cm sections per stem, the first from the crown and the second from the first node above the crown, were removed aseptically and plated on PPA. Plates were incubated and colonies purified and identified as for the wheat isolates.

Tab. 1. – Grass sampling site information including agroecological region, site number, location, grass genus and species.

Agroecological region	Site no.	Location	Grass species
West Coast of Marmara	1	Gumusyaka	*Avena sterilis* L.
	2	Gumusyaka	*Lolium multiflorum* Lam.
	3	Silivri	*Avena sterilis*
	4	Yenice	*Phalaris canariensis* L.
West Black Sea	5	Yarhisar	*Aegilops umbrelliata* Zhuk.
East Central Anatolia	6	Kayseri	*Aegilops triuncialis* L.
	7	Yozgat	*Hordeum geniculatum* All.

Results

Wheat

A total of 489 isolates representing 15 *Fusarium* species were recovered from 1250 wheat stem bases collected across 25 sites in northern Turkey. The species recovered included a number of well documented wheat pathogens, and a number of cosmopolitan secondary colonizers and saprobic species. The percentage isolation of each *Fusarium* species relative to total *Fusarium* recovery from each of the three agroecological regions is summarized in Tab. 2. The relative isolation frequencies for the eight most prevalent species across the three agroecological regions are summarized in Fig. 2, with the seven remaining *Fusarium* species being isolated only at low frequencies (Tab. 2). Additional putative isolates of the closely related *Microdochium nivale sensu lato* Samuels & Hallett (formerly classified as *F. nivale* (Fr.) Ces.) were isolated at a relatively high frequency from the west Coast of Marmara, but are not considered further in this study.

Tab. 2. – Percentage isolation of individual *Fusarium* species relative to total *Fusarium* recovery for each of the three agroecological regions: west Coast of Marmara, west Black Sea, and east Central Anatolia. Absence of a *Fusarium* species from a region is indicated by a blank cell.

	Agroecological Region		
***Fusarium* species**	West Coast of Marmara	West Black Sea	East Central Anatolia
F. oxysporum	33.2	64.6	31.3
F. equiseti	12.3	23.2	30.1
F. acuminatum	15.2	8.1	19.9
F. armeniacum	11.8		0.6
F. culmorum	11.8		0.6
F. torulosum	1.9	3.0	9.0

Tab. 2 continued.

***Fusarium* species**	**Agroecological Region** West Coast of Marmara	 West Black Sea	 East Central Anatolia
F. avenaceum	3.8		3.6
F. proliferatum	3.8		
F. reticulatum		1.0	2.4
F. compactum	1.9		0.6
F. pseudograminearum	0.9		
F. crookwellense	0.5		
F. solani			0.6
F. polyphialidicum			0.6
F. udum			0.6

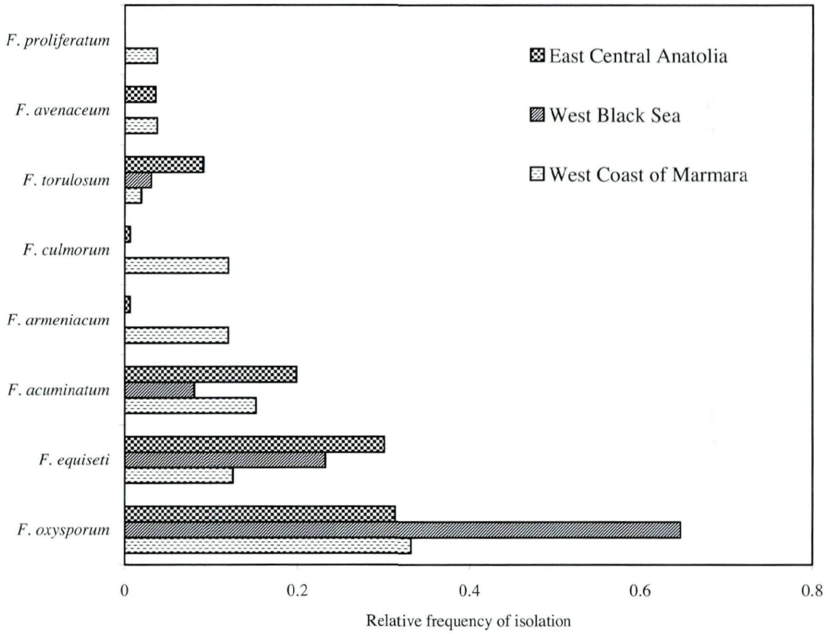

Fig. 2. Frequency of isolation of the eight most common species in the *Fusarium* complex from wheat stem bases from three agroecological regions of northern Turkey

A set of voucher specimens representing the range of *Fusarium* species isolated from wheat was deposited in the RBG collection at the Botanic Gardens Trust, Sydney [Species (RBG accession number/s)]: *F. oxysporum* (RBG5202, RBG5203), *F. equiseti* (RBG5204), *F. acuminatum* (RBG5205, RBG5206), *F. armeniacum* (RBG5207), *F. culmorum* (RBG5208, RBG5209, RBG5210, RBG5211), *F. torulosum* (RBG5212), *F. avenaceum* (RBG5213), *F. proliferatum* (RBG5214), *F. reticulatum* (RBG5215), *F. compactum* (RBG5216, RBG5217), *F. pseudograminearum* (RBG5218, RBG5219), *F. solani* (RBG5220), *F. polyphialidicum* (RBG5221) and *F. udum* (RBG5222).

The configuration of similarities of the *Fusarium* isolation data at all sites within the three agroecological regions as determined by non-metric multidimensional scaling using a Bray-Curtis similarity matrix is illustrated in Fig. 3, which indicates rough clustering of similarity within regions. Results of calculations of species diversity, richness and evenness are summarized in Tab. 3 and show the highest level of community diversity from the west coast of Marmara, followed by east Central Anatolia and the west Black Sea region.

At least one of the two key pathogens *F. culmorum* or *F. pseudograminearum* was isolated from 32 % of sites sampled. Percentage of pathogen isolation from sites within regions is summarized in Tab. 4, with *F. culmorum* most commonly isolated, present at 28 % of sites sampled, followed by *F. pseudograminearum* (8 % of sites). The range of these species at each site however was relatively low, ranging from 2 % to 16 % for *F. culmorum* and 2 % to 4 % for *F. pseudograminearum*.

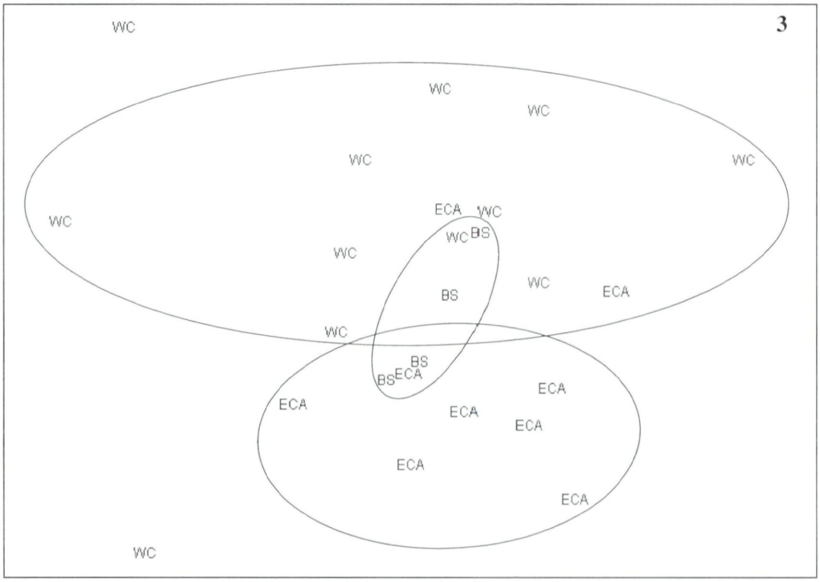

Fig. 3. Non-metric multidimensional scaling plot based on a Bray-Curtis similarity matrix on square root transformed isolation data. Overlayed ellipses indicate the rough clustering of community composition by agroecological region with a small number of outlying sites. WC: West Coast of Marmara, BS: Black Sea region and ECA: East Central Anatolia

Tab. 3. – Results of calculations for the diversity and range of species isolated from wheat stem bases in each agroecological region.

Agroecological region	S^a	d^b	J'^c	$1\text{-}\lambda'^d$
West Coast of Marmara	15	2.59	0.78	0.84
West Black Sea	6	1.09	0.57	0.54
East Central Anatolia	13	2.34	0.66	0.78

S^a = species richness
d^b = Margalef's index
J'^c = Pielou's evenness index
$1\text{-}\lambda'^d$ = Simpson's index

Tab. 4. – Percentage of sites within regions from which *F. culmorum* and/or *F. pseudograminearum* were isolated (percentage isolation frequency ranges for each species from individual sites within regions are given in brackets). All isolations were made from wheat stem bases collected randomly from arbitrarily selected sites in three agroecological regions of northern Turkey. Absence of a *Fusarium* species from a region is indicated by a blank cell.

Agroecological region	***F. culmorum***	***F. pseudograminearum***
West Coast of Marmara	50 (2 – 16)	17 (2 – 4)
West Black Sea		
East Central Anatolia	11 (2)	

The putative isolates of *F. pseudograminearum* clustered with designated reference strains with at least 80 % DICE similarity (data not shown). All putative *F. proliferatum* isolates produced fertile perithecia with standard tester strains confirming their identity as *F. proliferatum* (mating population D). Putative *F. torulosum* isolates grew at an average daily rate of 2.38 mm on PDA at 20 °C, and all isolates produced intercalary chlamydospores on SNA in the dark at 20 °C after 14 days, confirming their morphological identification as *F. torulosum* (Nirenberg 1995). The *Tef-1* sequences for the two randomly selected isolates clustered with the *F. torulosum* GenBank accessions with 100 % bootstrap support, further confirming their identity (data not shown).

Grasses

A total of twelve isolates representing five *Fusarium* species were obtained from six grass species collected at seven sites. Percentage of isolation for individual *Fusarium* species from each set of ten grass stems were calculated and are presented in Tab. 5.

The species isolated included the key wheat pathogens *F. culmorum, F. pseudograminearum,* and *F. graminearum.* All putative *F. pseudograminearum* isolates clustered with reference strains and all *F. graminearum* isolates produced fertile perithecia in cultures initiated from a single spore. A set of voucher specimens representing the range of *Fusarium* species isolated from grasses was deposited in the RBG collection at the Botanic Gardens Trust, Sydney [Species (RBG accession number/s)]: *F. culmorum* (RBG5233), *F. semitectum* (RBG5224), *F. graminearum* (RBG5225), *F. acuminatum* (RBG5226, RBG5227) and *F. pseudograminearum* (RBG5228).

Tab. 5. – Percentage isolation of *Fusarium* species from each of the seven grasses sampled from the west coast of Marmara (four sites, *A. sterilis, L. multiflorum, P. canariensis*), the west Black Sea region (one site, *Ae. umbellulata*) and east central Anatolia (two sites, *Ae. triunculatus, H. geniculatum*). Absence of a *Fusarium* species from a grass is indicated by a blank cell.

Grass species	Site/s	*F. culmorum*	*F. semitectum*	*F. graminearum*	*F. acuminatum*	*F. pseudograminearum*
A. sterilis	1, 3					
Ae. umbrelliata	5	10			20	
Ae. triunculatus	6				10	
H. geniculatum	7	10				10
L. multiflorum	2		10	10		
P. canariensis	4	20				

Discussion

The findings of the current study indicate that a relatively large *Fusarium* community is associated with wheat stem bases in three agroecological regions of northern Turkey. Two major soil-borne *Fusarium* pathogens of wheat, *F. culmorum* and *F. pseudograminearum* were recovered from the west coast of Marmara, with *F. culmorum* also being isolated from east central Anatolia. The foot rot pathogen *F. culmorum* was the most commonly isolated species in the Marmara region, indicating that the climate in this region may favor infection by, and survival of *F. culmorum*. The weaker pathogen *F. avenaceum* has also been shown to have a similar temperature adaptation as *F. culmorum* (Pettitt *et al.* 2003), and was isolated more commonly from the west coast of Marmara than the other two regions.

The presence of the foot rot pathogen *F. culmorum* from half of the sites sampled on the west coast of Marmara and one of the sites in east central Anatolia, albeit at a relatively low frequency from each site, indicates the need for further studies to clarify the importance of this pathogen. It is known that levels of *F. culmorum* have been gradually declining throughout cereal cropping regions of Europe, with this decline attributed to factors such as gradual shifts in climatic conditions, and displacement by other species (Ioos *et al.*

2004). The drift in *Fusarium* populations associated with head blight of cereals from *F. culmorum* to *F. graminearum* in recent years has been well documented, with *F. graminearum* thought to be increasingly favored as it has a higher temperature optimum for growth (Waalwijk *et al.* 2003). Further surveys will determine if levels of *F. culmorum* associated with stem base diseases in Turkey are also declining.

The crown rot pathogen *F. pseudograminearum* was detected at low levels and at present is unlikely to limit cereal production in the surveyed areas of northern Turkey. However, changes in cropping systems can dramatically alter the importance of particular species. The adoption of no-tillage farming systems in spring wheat areas in the Marmara region could greatly improve subsoil moisture retention (Evrendilek and Ertekin 2002), rectify soil degradation (Hall *et al.* 2005), and provide an economic and labor cost advantage (Ozpinar 2004). However, such systems could lead to an increase in the incidence of crown rot caused by *F. pseudograminearum* as has been seen throughout Australia (Burgess *et al.* 1993, Williams *et al.* 2002). It is also possible that the levels of *F. culmorum,* which are already much higher than those of *F. pseudograminearum,* could increase with the adoption of stubble retention.

Fusarium torulosum and *F. acuminatum* were isolated at relatively high levels from all three agroecological regions. This is consistent with the dominant isolation of these species from areas with Mediterranean-type climates characterized by cool to cold wet winters and hot, dry summers (Burgess *et al.* 1981, Ioos *et al.* 2004). It is speculated that these species are well adapted and distributed throughout northern wheat production regions of Turkey. The isolation of *F. armeniacum* from wheat stems on the west coast of Marmara represents the first report of this species in Turkey and is noteworthy due to its mycotoxigenic potential (Wing *et al.* 1993).

Fusarium equiseti and *F. oxysporum* were isolated at high frequencies from all three agroecological regions. The isolation of these species from randomly selected healthy, diseased or senescing tissue is consistent with their classification as cosmopolitan saprobic *Fusarium* species (Burgess *et al.* 1981). These species have both been previously documented in Turkey as weakly pathogenic colonizers of stem bases of wheat (Demirci and Dane 2003) and sainfoin (Eken *et al.* 2004), further justifying this classification. The presence of relatively high levels of typically weak or secondary pathogens in this study, and that of Demirci and Dane (2003) suggest that the aetiology of root and crown diseases in this area is complex, and that secondary colonization of diseased tissue is to be expected. This is a similar situation to that reported in wheat producing areas in the Pacific Northwest of the USA (Smiley and Patterson 1996), Texas (Specht and Rush 1988) Colorado and Wyoming (Hill *et al.* 1983) and South Australia (Williams *et al.* 2002).

Bray-Curtis analysis indicates that the composition of the *Fusarium* community varies across the broad agroecological regions studied, although there is some overlap in species composition. Calculations of Simpson's Index of Diversity, species richness and species evenness also revealed differences between the regions. The west coast of Marmara exhibited the highest diversity of species, followed by east central Anatolia, and then the west Black Sea region. High diversity in the Marmara region is thought to be influenced by climatic conditions, notably mild winters, and above average rainfall, which are speculated to be conducive to high levels of *Fusarium* diversity. The relatively low number of *Fusarium* species and level of diversity recorded from the west Black Sea region may be attributed to the limited number of samples from this region. Freezing winter temperatures and high altitude may also explain lower levels of species diversity and the recovery of predominantly saprobic, chlamydospore forming *Fusarium* species in this region.

The wheat pathogens *F. culmorum*, *F. pseudograminearum* and *F. graminearum* were found to be associated with grass stem bases in Turkey in the current study. *Fusarium culmorum* was associated with half of the grass species examined, indicating that it is present in non-cropping grasslands as well as in cropping systems, and it is speculated that *F. culmorum* is endemic in northern Turkey. This is further supported by the isolation of *F. culmorum* from *Aegilops umbellulata,* which occurs predominantly in Turkey, and is the contributor of the common U-genome to all polyploid species in the section Aegilops (Kawahara 2002) including *A. tauschii* Coss., a progenitor of modern hexaploid bread wheat. These preliminary data are significant as wild species of the wheat group are useful sources of economically significant genetic material, such as disease resistance genes, for cultivated wheat breeding programs (Kawahara 2002). Further studies are required to more completely document the associations of *Fusarium* species with grasses in northern Turkey.

The use of a comprehensive survey to collect data from arbitrarily selected sites within large agroecological regions and to select plants on a random basis within individual fields has allowed an insight into the relative frequency and distribution of *Fusarium* species in northern Turkey. Despite the presence of a relatively diverse *Fusarium* community associated with wheat and grass stem bases, there is little quantitative evidence to suggest that these species are a major factor limiting wheat production in northern Turkey despite anecdotal evidence suggesting otherwise. In cases where disease is seen, *Fusarium* pathogens may be isolated, but at present it appears that random sampling does not allow us to readily anticipate key disease or pathogen areas as underlying pathogen frequencies exist at relatively low levels. Although there is little evidence to suggest that pathogenic *Fusarium* species are widespread on wheat stem bases in Turkey, it is important to recognize the current range and frequency of *Fusarium* species present. These species should be monitored over time, and in association with visible manifestations of disease in order to make decisions regarding the direction of crop management and *Fusarium* disease control in northern Turkey.

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