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Short communication

Gramineous and non-gramineous weed species as alternative hosts of *Fusarium graminearum*, causal agent of Fusarium head blight of wheat, in Argentina

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ABSTRACT

Weeds and wild plants around and within crops could serve as alternative hosts of fungal pathogens. In this work we describe the isolation of *Fusarium graminearum*, the main causal agent of Fusarium head blight (FHB) in Argentina from the inflorescences of healthy weed plants belonging to sixty seven gramineous and non-gramineous species, which showed no symptoms of *Fusarium* infection, sampled throughout a year. Fifty four of the weed species considered, belonging to 19 botanical families, were first identified as alternative hosts of *F. graminearum* in the present work. Furthermore, the trichothecene chemotype of a group of isolates was analysed and strains belonging to 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and nivalenol chemotypes were found. The information provided could prove valuable to study further the epidemiological role of weeds in FHB epidemics, which might help to improve management of the disease in wheat growing areas.

Fusarium graminearum Schwabe [teleomorph *Gibberella zeae* (Schwein.)Petch], is the main causal agent of Fusarium head blight (FHB) of wheat and other small grains in Argentina (De Galich, 1997; Lori et al., 2003).

The pathogen damages yield and quality of the grain, mainly because of its frequent contamination with mycotoxins which constitute a risk for human and animal health (Kendrick, 1992). *F. graminearum* produces one of three sets of type-B trichothecene metabolites, nivalenol (NIV) and its acetylate derivative fusarenone X (FUS-X) (NIV chemotype), deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-ADON chemotype) or DON and 15-acetyldeoxynivalenol (15-ADON chemotype) (Ward et al., 2002). Different authors reported the prevalence of either DON or NIV chemotypes according to the geographic distribution of *F. graminearum* (Wang et al., 2011). In Argentina, although 15-ADON is the most common chemotype (Alvarez et al., 2009; Malbrán et al., 2014), the presence of NIV chemotype has also been reported (Lori et al., 1992; Fernández Pinto et al., 2008; Sampietro et al., 2011; Reynoso et al., 2011).

Environmental conditions, mainly spike wetness duration and temperature, have been indicated as a key factor for the development of FHB epidemics (Moschini and Fortugno, 1996). Additionally, as FHB is a monocyclic disease (Landschoot et al., 2011), the quantity of primary inoculum available for infection could be of importance. However, under favourable climatic conditions, a rather small amount of inoculum might be enough to develop an epidemic (Dill-Macky and Jones, 2000; Miller et al., 1998).

Several structures produced by *F. graminearum* can act as inoculum for FHB, including macroconidia produced by saprophytic mycelia overwintering in the residues of several crops (Sutton, 1982; Bai and Shaner, 1994; Goswami and Kistler, 2004) as well as ascospores released by the pathogen from crop residues (Gilbert and Fernando, 2004; Dufault et al., 2006; Stein et al., 2009).

Recently, the host range of *F. graminearum* has expanded from cereal to non-cereal crops, including soybean (*Glycine max* L.) (Pioli et al., 2004; Broders et al., 2007). A host is defined as “a living organism harbouring a parasite” (Ainsworth, 1971) and under this definition are included both the economically important main hosts and potential alternative hosts. Several weed species have

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been identified as alternative hosts of *F. graminearum* and their possible role as inoculum sources for the development of FHB has been proposed (Jenkinson and Parry, 1994; Carmona et al., 1999; Inch and Gilbert, 2003; Pereyra and Dill-Macky, 2008; Landschoot et al., 2011; Postic et al., 2012; Tekle et al., 2012). Even though crop residues have been identified as the primary source of inoculum for the disease, the role weeds play in the epidemiology of FHB, especially non-gramineous species, remains an important aspect to be clarified and might lead to the development of management practices aimed at reducing their impact as inoculum sources. The objective of this work was to analyse the colonization of inflorescences of weeds belonging to different botanical families collected in the proximity of cropping areas by *F. graminearum* to establish their potential role as alternative hosts of the fungus.

Healthy, symptomless weed plants were randomly collected in the neighbourhood of soft wheat (*Triticum aestivum* L.) durum wheat (*Triticum durum* Desf.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), rye [*Secale cereale* (L.) M. Bieb.] and maize (*Zea mays* L.) crops. The assayed areas were located in the Estación Experimental Julio Hirschhorn of the Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Provincia de Buenos Aires, Argentina (34° 52′ S y 57° 58′ W). During a one year period, six sampling events were conducted (February, April, June, September and November 2010 and January 2011).

A total of 162 gramineous and non-gramineous weed samples were collected: 23 in February, 32 in April, 23 in June, 20 in September, 28 in November and 36 in January. Plants were taken to the laboratory, air dried, placed in paper envelopes and stored at 4 °C until they were processed. Weed species were identified according to their morphology by means of the identification keys by Cabrera and Zardini (1993).

Fusarium spp. isolates were obtained from whole weed inflorescences, which were dislodged from the plant. Larger inflorescences were cut into 0.5–2 cm pieces, each comprising at least one whole flower. Twelve pieces per plant were superficially disinfested by dipping in 70% ethanol for 1 min and in a 5% commercial NaClO solution (55 g of Cl L^{-1}) for 1 min, followed by rinsing in sterile distilled water for 5 min. Surface-sterilized pieces of inflorescences were plated on two 9 cm Petri dishes (6 pieces per plate) containing potato dextrose agar medium (PDA) 2% (w/v) supplemented with 250 mg L^{-1} of chloramphenicol and 600 mg L^{-1} of pentachloronitrobenzene (PCNB 75% wettable powder). After 5–7 days of incubation at 25 °C ± 2 °C, the incidence of *F. graminearum* colonization (number of inflorescence pieces colonized over the total) was evaluated for each weed species and sampling event. Isolates of *Fusarium* spp. were transferred to PDA plates, incubated at 25 °C ± 2 °C for 7 days and identified based on the cultural features and morphology of spores and conidiogenous cells (Booth, 1971; Leslie and Summerell, 2006).

The identity of the isolates was confirmed by running a species specific PCR using primer pair Fg16NF (5′-ACA GAT GAC AAG ATT CAG GCA CA-3′) and Fg16NR (5′-TTC TTT GAC ATC TGT TCA ACC CA-3′) developed by Nicholson et al. (1998) according to a modification of the procedure described by Malbrán et al. (2012).

Isolates identified as *F. graminearum* from 49 of the weed species considered were randomly chosen and their chemotype was analysed by means of the chemotype-specific multiplex PCR developed by Starkey et al. (2007). Primers 12NF (5′-TCT CCT CGT TGT ATC TGG-3′), 12–15F (5′-TAC AGC GGT CGC AAC TTC-3′), 12-3F (5′-CTT TGG CAA GCC CGT GCA-3′) and 12CON (5′-CAT GAG CAT GGT GAT GTC-3′), targeting the *TRI12* gene, were used according to the procedure described by Malbrán et al. (2014).

Data taken as percentage were arcsine-transformed prior to analysis. The incidence of *F. graminearum* colonization of weed inflorescences in each of the sampling events was analysed by analysis of variance (ANOVA) and means were compared using Tukey's test ($P = 0.05$). The analyses were performed using Statistix v.8 sofware (Analytical Software, Tallahassee, USA).

Sixty seven species of weeds, belonging to 22 botanical families, were collected in 6 sampling events that were distributed during a year (Table 1). From the inflorescences of the 162 weed samples analysed, 1020 isolates of *F. graminearum* were obtained and identified by means of morphological and molecular features. Although other fusaria were collected from the inflorescences, *F. graminearum* was the prevalent species and was found colonizing every weed species tested. More than two thirds of the 67 weed species colonized by *F. graminearum* were non-gramineous.

None of the weed species analysed presented symptoms of *Fusarium* infection, in agreement with previous reports (Jenkinson and Parry, 1994; Inch and Gilbert, 2003). In this regard, an endophytic colonization by *F. graminearum* on alternative hosts has been suggested by Sieber et al. (1988). Moreover, Brown et al. (2010) demonstrated that during an asymptomatic stage of FHB development, *F. graminearum* hyphae can grow in the intercellular spaces within spikes feeding exclusively from the extracellular exudates.

The chemotype of 107 *F. graminearum* isolates obtained from 49 weed species was determined. Ninety six isolates (89.7%) belonged to the 15-ADON chemotype, 2 (1.9%) to the 3-ADON and 9 (8.4%) to the NIV chemotype (Table 1). In Argentina, evidence suggests that the predominant trichothecene among *F. graminearum* populations is DON (Dalcero et al., 1997; Lori et al., 2003; Ramírez et al., 2006; Alvarez et al., 2009; Reynoso et al., 2011; Malbrán et al., 2014), even though NIV producers have been found (Lori et al., 1992; Fernández Pinto et al., 2008). In isolates obtained from wheat, 15-ADON producers prevailed (Alvarez et al., 2009; Reynoso et al., 2011; Malbrán et al., 2014) over those producing 3-ADON (Ramírez et al., 2006; Alvarez et al., 2009) and NIV (Lori et al., 1992; Fernández Pinto et al., 2008). In maize, on the other hand, an important presence of NIV producing isolates has been reported (Sampietro et al., 2011). The higher proportion of 15-ADON found in this work agrees with these reports. These results suggest that potential exists for the contamination of food and feeds with an important range of trichothecenes originated by the infection of economically important crops by *F. graminearum* isolates from weed species.

Thirteen weed species from 6 botanical families (Asteraceae, Chenopodiaceae, Convolvulaceae, Plantaginaceae, Poaceae and Polygonaceae) sampled in our work were previously cited as hosts of *F. graminearum* in Argentina (Carmona et al., 1999), Canada (Inch and Gilbert, 2003), Croatia (Postic et al., 2012), England (Jenkinson and Parry, 1994) and Uruguay (Pereyra and Dill-Macky, 2008). The presence of the fungus was reported on rhizomes and roots of plants of these weed species (Carmona et al., 1999; Postic et al., 2012). In this work, on the other hand, all the isolates were obtained from inflorescences. The colonization of weed inflorescences by *F. graminearum* could prove to be more relevant from an epidemiological point of view as FHB develops on the aerial organs of wheat. Fifty four weed species and 16 botanical families are reported as alternative hosts for the first time here (Table 1).

Weed species considered in our work had different growth habits and life cycles and as a consequence the species collected at each sampling event were different. Of the 67 weed species found, only *Cynodon dactylon* (L.) Pers., *Cyperus eragrostis* Lam., *Picris echioides* L., *Raphanus sativus* L., and *Dactylis glomerata* (L.) were present at all sampling events. Even though *F. graminearum* was found colonizing weed inflorescences throughout the year, the incidence of colonization in the different seasons was significantly different ($F = 4.35$; $p < 0.01$). The highest incidences of colonization were found in the summer (69% in February 2010) and spring seasons (58% in November 2010) while the lower incidences corresponded to the autumn (45% in April 2010) and winter seasons

Table 1
List of families, species and habits of weeds sampled for isolation of *Fusarium graminearum* and chemotype of isolates identified by Polymerase Chain Reaction (PCR).

Family	Weed species	Habit	Chemotype		
			15-ADON	3-ADON	NIV
Amaranthaceae	*Alternanthera philoxenoides* (Mart.) Griseb[a]	Perennial	n.d.	n.d.	n.d.
	Amaranthus dubius Mart. Ex Thell[a]	Annual	−	−	+
Apiaceae	*Cyclospermum leptophyllum* (Pers.) Sprague[a]	Annual	+	−	−
	Foeniculum vulgare Mill[a]	Perennial	+	−	−
Apocynaceae	*Oxypetalum solanoides* Hook. and Arn.[a]	Perennial	n.d.	n.d.	n.d.
Asteraceae	*Anthemis cotula* L.[a]	Annual	n.d.	n.d.	n.d.
	Aster squamatus (Spreng.) Hieron.[a]	Perennial	+	−	−
	Baccharis glutinosa Pers.[a]	Perennial	n.d.	n.d.	n.d.
	Bidens subalternans DC[a]	Annual	n.d.	n.d.	n.d.
	Cichorium intybus L.[a]	Perennial	−	−	+
	Cirsium arvense (L.) Scop.[a]	Annual	n.d.	n.d.	n.d.
	C. vulgare (Savi) Ten.[a]	Biennial	n.d.	n.d.	n.d.
	Coniza bonariensis (L.) Cronquist[a]	Annual	+	−	−
	Galinsoga parviflora Cav.[a]	Annual	+	−	−
	Gamochaeta spicata (Lam.) Cabrera[a]	Annual/biennial	+	−	−
	Hypochaeris radicata L.[a]	Perennial	n.d.	n.d.	n.d.
	Matricaria recutita L.	Annual	+	−	−
	Picris echioides L.[a]	Annual	+	−	−
	Solidago chilensis Meyen[a]	Perennial	+	−	−
	Sonchus asper (L.) Hill[a]	Annual	+	−	−
	S. oleraceus L.[a]	Annual	+	−	−
	Taraxacum officinale G. Weber ex F. H. Wigg[a]	Perennial	n.d.	n.d.	n.d.
Boraginaceae	*Echium plantagineum* (L.)[a]	Annual	+	−	−
Brassicaceae	*Brassica rapa* L.[a]	Annual/biennial	+	−	−
	Capsella bursa-pastoris (L.) Medik.[a]	Annual	n.d.	n.d.	n.d.
	Raphanus sativus L.[a]	Annual/biennial	+	−	+
Cariophyllaceae	*Cerastium glomeratum* Thiull.[a]	Annual	n.d.	n.d.	n.d.
Chenopodiaceae	*Chenopodium hircinum* Schrad.[a]	Annual	+	−	−
	C. quinoa Wild.	Annual	+	−	−
Convolvulaceae	*Convolvulus arvensis* L.	Perennial	+	−	−
Cyperaceae	*Cyperus eragrostis* Lam.[a]	Perennial	+	−	−
	C. rotundus (L.)[a]	Perennial	+	−	−
Fabaceae	*Trifolium pratense* L.[a]	Perennial	+	−	−
	T. repens L.[a]	Perennial	−	−	+
	Vicia villosa Roth[a]	Annual	n.d.	n.d.	n.d.
Fumariaceae	*Fumaria capreolata* L.[a]	Annual	+	−	−
Gentianaceae	*Centaurium pulchellum* (Sw.) Druce[a]	Annual	n.d.	n.d.	n.d.
Lamiaceae	*Lamium amplexicaule* L.[a]	Annual	+	−	−
	Stachys arvensis (L.) L.[a]	Annual	+	−	+
Malvaceae	*Anoda cristata* L.[a]	Perennial	+	+	−
Plantaginaceae	*Veronica persica* Poir.	Annual	+	−	−
Poaceae	*Avena byzantina amarilla* C. Koch[a]	Annual	n.d.	n.d.	n.d.
	A. fatua L.[a]	Annual	+	−	−
	A. sativa L.	Annual	−	−	+
	Bothriochloa laguroides (DC.) Herter[a]	Perennial	n.d.	n.d.	n.d.
	Briza minor L.[a]	Annual	n.d.	n.d.	n.d.
	Bromus mollis L[a].	Annual	+	−	−
	B. unieloides Kunth	Perennial	+	−	−
	Cynodon dactylon (L.) Pers.	Perennial	+	−	−
	Dactylis glomerata (L.)[a]	Perennial	+	−	+
	Digitaria sanguinalis (L.) Scop.	Annual	+	−	−
	Echinochloa crus-galli (L.) P. Beauv	Annual	+	−	−
	Festuca sp.[a]	Perennial	+	−	−
	F. arundinacea Schreb.	Perennial	+	−	−
	Lolium multiflorum Lam.	Annual/biennial	+	−	+
	Panicum sp.[a]	Annual	+	−	−
	Paspalum dilatatum Poir.[a]	Perennial	+	+	−
	Phalaris aquatica L.[a]	Perennial	−	−	+
	Sorghum halepense (L.)Pers.	Perennial	+	−	−
Polygonaceae	*Rumex crispus* L.	Perennial	+	−	−
Portulacaceae	*Portulaca oleracea* L.[a]	Annual	+	−	−
Rubiaceae	*Borreira verticillata* (L.) G. Mey[a]	Annual/perennial	n.d.	n.d.	n.d.
Solanaceae	*Datura ferox* L.[a]	Annual	+	−	−
	Nicotiana longiflora Cav.[a]	Perennial	+	−	−
	Solanum chenopodioides Lam.[a]	Annual	n.d.	n.d.	n.d.
Verbenaceae	*Verbena montevidensis* Spreng.[a]	Perennial	+	−	−
	V. bonariensis L.[a]	Annual/biennial	+	−	−

n.d.: Not determined.
[a] First report of *F. graminearum* isolation on this host.

(46% in June 2010). A similar trend was found when only the 5 species present in the 6 sampling events were analysed, even if the differences found were not significant ($F = 2.11$, $P = 0.0992$).

In Argentina's Pampas Region, weather conditions favour the abundance and prevalence of weed plants surrounding and inside the cropping fields throughout the year. Such temperate weather conditions and the ample range of weed species hosting *F. graminearum* that had different growing habit might explain the ubiquitous presence of the pathogen.

Alternative hosts have been cited as important epidemiological bridges that allow for pathogen overseasoning, increasing or maintaining the amount of inoculum against the natural tendency for decrease in the absence of crops of the main host (Dinoor, 1974). Inch and Gilbert (2003) proposed that, due to the lack of perithecia of *G. zeae*, wild grasses would not contribute the ascospores that serve as initial inoculum for the development of FHB but could serve as reservoir for the pathogen in the fields when no susceptible hosts were present (Carmona et al., 1999; Inch and Gilbert, 2003). However, Pereyra and Dill-Macky (2008) reported the production of *G. zeae* perithecia on gramineous weed residues.

In Argentina, recent surveys indicate that 27 million hectares of crops (78.5% of the total arable land in the country) are seeded under no tillage (Trigo et al., 2009; AAPRESID, 2012), which leaves a high amount of crop as well as weed residues on the soil surface. Under these conditions, alternative hosts of *F. graminearum* might not only act as a reservoir of the pathogen between two susceptible crops but also produce *G. zeae* perithecia on their residues that could in time serve as inoculum sources for initial FHB infection. Even if *G. zeae* produces fewer ascospores on weeds than on crop residues (Pereyra and Dill-Macky, 2008), its contribution could prove to be important under favourable weather conditions (Lori et al., 2009).

The information provided in this work could prove valuable to better understanding the epidemiology of *F. graminearum* and for the development of management practices aimed at reducing the amount of inoculum available for FHB epidemics. However, further research is necessary to accurately define the impact of weeds as alternative hosts. Future work should focus on the precise determination of the infection mechanism employed by the fungus in its interaction with weed plants as well as the type of inoculum provided by spontaneous species and its importance for the epidemiology of FHB. In this regard, work is being conducted to evaluate the pathogenicity of the *F. graminearum* strains recovered from weed inflorescences.

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