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Regional and varietal differences in the risk of wheat seed infection by fungal species associated with fusarium head blight in Italy

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Abstract

The incidence of seed infection by fungal species pertinent to the fusarium head blight complex was monitored from 1999 to 2002 in two soft and three durum wheat cultivars grown across the northern, central and southern production zones of Italy, in order to characterize the species composition at the seed level. The main species recovered were *Fusarium graminearum*, *F. poae* and *Microdochium nivale*. There was a marked influence of production zone on seed infection incidence for both durum and soft wheat cultivars, with incidence of infection generally decreasing from the northern to the southern zone. Incidence of seed infection by different species of *Fusarium* was twice to four times higher in durum compared with the soft wheat cultivars in the study. There were no significant differences in terms of seed infection incidence between the two soft wheat cultivars, but the durum cultivars differed in their levels of seed infection for some of the pathogens. The results demonstrated that the durum cultivars were more at risk of seed infection by pathogens associated with fusarium head blight, and that wheat grown in northern Italy is at higher risk of seed infection by these species.

Introduction

Fusarium head blight (FHB) is mainly associated with five species (Parry et al., 1995): *F. avenaceum*, *F. culmorum*, *F. graminearum* (teleomorph: *Gibberella zeae*, *F. poae*, and *Microdochium nivale* (syn.: *F. nivale*). The relative importance of the different species can vary with climate, the agronomy of different regions, and also temporally (Bottalico and Perrone, 2002). For example, FHB is caused predominantly by *F. graminearum* in North America (Gilbert and Tekauz, 2000), Australia (Akinsanmi et al., 2004), and China (Wang, 1997). However, *F. avenaceum* and *F. poae* are predominant in some European countries (Schilling et al., 1997; Kosiak et al., 2003), and *F. culmorum* was the major species associated with FHB in the United Kingdom prior to 1995 (Parry et al., 1995).

Characterizing the species associated with FHB, either on spikes or within seed, is a necessary first step in the cumulative research effort for effective disease control strategies. This initial effort is usually met by surveys to document disease prevalence and intensity (Del Ponte et al., 2003), and provides the foundation for further research into other aspects of a disease. Surveys of disease occurrence range in complexity from simply random to fairly complex designs, and the reported results likewise vary from the simple documentation of prevalence to the spatiotemporal modelling of georeferenced incidence data to ascertain underlying causative factors. In the plant pathological literature, survey data are typically reported as summaries of mean incidence (or severity) by cultivar, region or year, and may include efforts to correlate disease level with environmental factors. The prevalence or incidence of plant diseases can however vary quite markedly from year to year, or from one region to the next, making it difficult to discern the longer-term trends in disease occurrence, or patterns which vary geographically. Attempts to discover such longer-term trends have been made recently by applying mixed model theory and software to data collected over several seasons or regions (Gladders et al., 2001; Shah et al., 2002b; Shah and Stivers-Young, 2004). Although the general consensus is that FHB incidence is affected by climate on the larger geographic scale (as opposed to the impact of environmental conditions at the local scale), there have been few attempts to quantify this effect (Backhouse and Burgess, 2002). In this paper, we apply generalized linear mixed models (Littell et al., 1996) to quantify the trends in wheat seed infection by fungal species associated with FHB in five cultivars across Italian production zones. Preliminary results have been published (Infantino et al., 2003).

Materials and methods

Experimental plots

Plots of two soft wheat (*Triticum aestivum*) cultivars (Centauro and Eridano), and three durum wheat (*Triticum durum*) cultivars (Appulo, Simeto and Zenit) were sown at several sites across the northern, central and southern regions of Italy from 1999 to 2002 (Table 1). At each site, the plots followed a randomized complete block design with two replicates (blocks) per cultivar. Plots were sown at 450 germinating seeds per m^2. Each plot was 10 m^2 (1.5 m by 7 m) and consisted of 8 rows spaced 17–19 cm apart.

The cultivars chosen were representative of the more susceptible ones common in Italian production. Soft wheat is grown predominantly in the Emilia-Romagna and the surrounding northern regions. Durum cultivation is concentrated in the southern regions, with the Sicilia and Puglia regions combined accounting for about 50% of the total durum production. Both soft and durum wheat are sown around the same time, beginning around October 1st and continuing to the end of November. Soft wheat harvest occurs between July 1st and August 31st. Durum is harvested slightly earlier, beginning around June 1st and continuing until the end of July.

Fungal identification

Seed was harvested with a small plot combine with fan speed set low to prevent light-weight, potentially infected kernels from being blown out. Seed were stored at 4 °C until assay. Subsamples of 100 seeds from each plot were assayed for seedborne fungi by the freezing blotter method (Limonard,

Table 1. Locations across Italy in which cultivars were evaluated for seed infection by *Fusarium*

Site	Zone	Longitude	Latitude	1999	2000	2001	2002
Caleppio di Settala (MI)	North	9.3882 E	45.4545 N		×		×
S.Angelo Lodigiano (LO)	North	9.4097 E	45.2390 N	×	×	×	×
Gariga di Podenzano (PC)	North	9.6860 E	44.9573 N		×	×	×
Imola	North	11.7168 E	44.3532 N	×			×
Arezzo	Center	11.8700 E	43.4733 N	×	×	×	×
S. Apollinare	Center	12.3072 E	43.0041 N		×	×	×
Papiano	Center	12.3766 E	42.9569 N		×	×	×
Spoltore (PE)	Center	14.1419 E	42.4500 N	×			×
Villareia di Cepagatti (PE)	Center	14.0767 E	42.3600 N		×	×	
Montelibretti (RM)	Center	12.7396 E	42.1365 N		×	×	×
Larino (CB)	Center	14.9202 E	41.8065 N	×	×	×	×
Foggia	South	15.5501 E	41.4619 N	×	×	×	×
Spinazzola (BA)	South	16.0924 E	40.9681 N	×			
Poggiorsini (BA)	South	16.2556 E	40.9181 N		×		×
Gravina (BA)	South	16.4236 E	40.8207 N			×	
Caltagirone (CT)	South	14.5164 E	37.2187 N			×	
Ispica (RG)	South	14.9055 E	36.7844 N		×		

1966). Identification of *Fusarium* species was based on the morphology of macroconidia under the compound microscope (magnification 10–100×) following the criteria of Nelson et al. (1983). Colonies were also plated on carnation leaf agar when identification could not be confirmed by the initial microscopic examination alone. Data were recorded as the number of seeds per hundred assayed positive for the given species of *Fusarium*.

Statistical analysis

The process of choosing an appropriate statistical model involves some preliminary understanding of the nature of the dataset. In the current study, the assessed variable (seed infection incidence) is a binary response (either a seed is infected or not) measured on subsamples of 100 seeds. The dataset is unbalanced, as not all locations were represented in each of the study years (Table 1). In addition to the expected variation among years, preliminary summaries of the data suggested a general decrease in seed infection incidence from the northern to southern zones for both durum and soft wheat cultivars. Moreover, the observed means and variances in seed infection incidence were well described by the binary power law (Hughes and Madden, 1992), demonstrating that the variance increased with the mean (Figure 1), a phenomenon previously observed with *F. graminearum* in wheat seed (Shah et al., 2002a).

Given the underlying structure of the dataset, seed infection incidence data were analyzed using a generalized linear mixed modelling approach:

$$\mathrm{logit}(\pi_{ijk(l)}) = \alpha_i + \beta_j + \alpha\beta_{(ij)} + \gamma_{k(l)} + \alpha\gamma_{ik(l)} + \epsilon_{ijk(l)} \qquad (1)$$

where $\mathrm{logit}(\pi_{ijk(l)})$ is defined as $\log[\pi_{ijk(l)}/(1-\pi_{ijk(l)})]$, modelled as a linear combination of the effects of the *i*th cultivar, the *j*th zone, the *k*th location (within the *l*th year), and $\varepsilon_{ijk(l)}$ is the normal residual error associated with the aforementioned effects. Each specific location within a given year ($\gamma_{k(l)}$) was considered a random effect (i.e. we considered each location by year combination as unique). Interactions between locations and cultivar were also treated as random effects.

One advantage of mixed models is their ability to account for heterogeneity in the variances directly in the model specification. This was done with the current datasets by specifying separate covariance structures in the $\varepsilon_{ijk(l)}$ for each of the three zones. Models were implemented using the glimmix macro (Littell et al., 1996) of SAS (SAS Institute, Cary, NC).

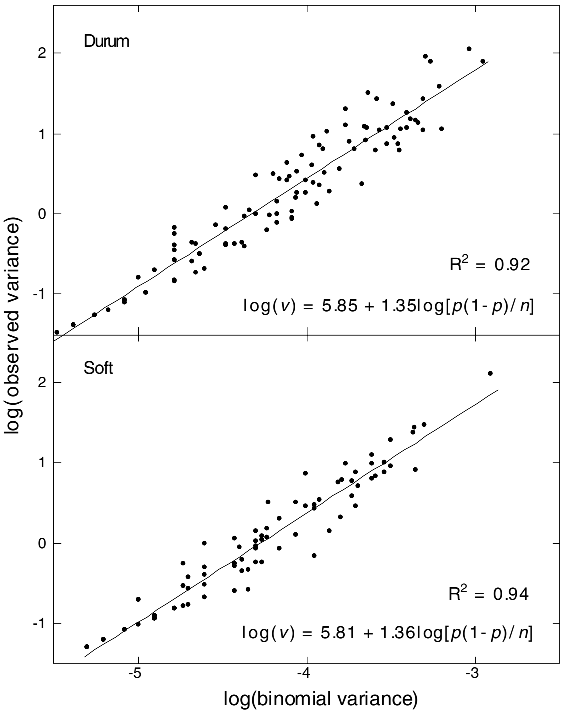

Figure 1. Relationship between the log of the observed variance in seed infection incidence (v) and the log of the variance assuming seed infection incidence follows a binomial distribution. The equations give the fit of the data to the linearized form of the binary power law (Hughes and Madden, 1992), where p is the estimate of the mean seed infection incidence and n is the number of seeds assessed for infection by a particular *Fusarium* species ($n = 100$ in this study). Data were plotted separately for durum and soft wheats, grown in experimental survey plots across three zones in Italy from 1999 to 2002. Each point represents a single year × zone × *Fusarium* species combination.

Weather data (precipitation, minimum and maximum temperatures) recorded at three representative stations, Lodi (North), Arezzo (Centre), Foggia (South) were examined for general trends over the mean flowering to early grain filling period and were compared with longer-term climatological records.

Results

Fungal identification

Several species of *Fusarium* were identified: *F. graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum*, *F. subglutinans*, *F. equiseti*, *F. verticillioides*, *F. proliferatum*, *F. semitectum*; as well as *Microdochium nivale*.

Effect of wheat type

A comparison of incidence of seed infection by the different species of *Fusarium* was made between soft and durum wheats across the three regional zones. Mean seed infection incidence was below 3%, with *F. graminearum*, *F. poae* and *M. nivale* generally being the most common species (Table 2). For most of the species there were significant differences among the regional zones in terms of seed infection incidence, but there were also significant differences between the soft and durum wheat groups. Odds ratios showed that the durum cultivars were more likely to harbour a higher incidence of seed infection than the soft wheat cultivars (Table 2). Therefore, soft and durum wheat data were analyzed separately.

Soft wheat

F. graminearum, *F. poae* and *M. nivale* were the most common species found in the seeds of the soft wheat cultivars Centauro and Eridano. The incidence of seed infection by the various species was not significantly different ($p > 0.05$) between the two cultivars, but there were significant differences among zones in terms of seed infection (Table 3). Generally, seed infection incidence was highest in the northern zone and lowest in the southern zone.

Durum wheat

The predominant species recovered were also *F. graminearum*, *F. poae* and *M. nivale* (Table 4). As found with the soft wheat cultivars, seed infection incidence in the durum cultivars varied by zone. For example, seed infection incidence by *F. graminearum* was three times higher in the northern zone compared with the central zone, and 36 times higher than the mean incidence for the southern zone. The same trend was seen for seed infection by *M. nivale*. In general, seed infection incidence was lowest in the southern zone (Table 4).

There were also significant differences among the cultivars in seed infection incidence for some of the *Fusarium* species. For example, the incidence of seed infection by *F. poae* was significantly lower in CV. Appulo compared with either Simeto or Zenit.

General regional climate trends

Over the four years of the study, there was a general north to south decrease in rainfall during the flowering and early grain fill periods (Table 5),

Table 2. Statistical *p* values for tests of differences among regional zones (north, central, south) and wheat type (durum, soft) in incidence of seed infection by various species of *Fusarium* in Italy, 1999–2002

Pathogen	*p* value			Seed infection incidence (%)		
	Wheat Type	Zone	WT*Z[a]	Durum	Soft	Odds Ratio[b]
F. avenaceum	0.0010	0.0002	0.0184	0.77	0.47	1.64
F. culmorum	0.0038	0.0311	0.0326	0.30	0.08	3.64
F. equiseti	0.0533	0.0011	0.0466	0.42	0.29	1.44
F. graminearum	0.0229	< 0.0001	0.0581	1.55	1.16	1.33
F. poae	0.0002	0.0121	0.8729	2.01	1.01	2.01
F. proliferatum	0.3183	0.2422	0.1451	0.18	0.12	1.51
F. semitectum	0.0005	0.0027	0.0712	0.71	0.41	1.73
F. subglutinans	0.0203	0.1361	0.7700	0.29	0.10	2.76
F. verticillioides	0.0011	0.0058	0.8167	1.03	0.58	1.80
M. nivale	< 0.0001	< 0.0001	< 0.0001	1.92	0.95	2.03
Fusarium spp.[c]	0.0515	0.0065	0.0061	0.46	0.27	1.67

[a]Wheat type (WT) by regional zone (Z) interaction.
[b]The odds ratio measures the increased risk of seed infection for the durum wheats evaluated in this study compared with the soft wheats. The odds ratio (Ψ) is given by $\Psi = [p_d(1-p_d)]/[p_s(1-p_s)]$, where p_s is the mean probability of seed infection by the particular *Fusarium* species for the soft wheats, and p_d is the mean probability of seed infection for the durum wheats.
[c]Species of *Fusarium* that were not one of the above (i.e. rare species), or which could not be identified, were classified as *Fusarium* spp.

Table 3. Mean seed infection incidence by *Fusarium* species for two soft wheat cultivars grown in three regional zones (north, central, south) in Italy, 1999 to 2002. Contrasts among the regional zones are shown

Pathogen	*p* value			Incidence of seed infection (%)[a]					Contrast *P* value[c]		
				By cultivar		By zone					
	Cultivar	Zone	C*Z[b]	Centauro	Eridano	North	Central	South	C–N	C–S	N–S
F. avenaceum	.[d]	.	.	0.56	0.76	1.23	0.87	0.00	.	.	.
F. culmorum	0.2561	0.2901	0.2586	0.12	0.05	0.18	0.04	0.04	0.1448	0.7996	0.1183
F. equiseti	0.5954	0.0004	0.3582	0.40	0.31	0.97	0.22	0.04	0.0120	0.1084	0.0003
F. graminearum	0.6578	< 0.0001	0.8470	1.08	1.17	4.01	0.70	0.12	0.0002	0.0851	< 0.0001
F. poae	0.1291	0.0112	0.7465	1.10	0.78	1.14	1.57	0.32	0.4430	0.0035	0.0462
F. proliferatum	0.1081	0.1542	0.2158	0.20	0.08	0.15	0.22	0.05	0.5767	0.0755	0.2293
F. semitectum	0.8457	0.0018	0.6300	0.45	0.47	0.88	0.55	0.07	0.2186	0.0131	0.0007
F. subglutinans	0.3238	0.0646	0.4666	0.12	0.09	0.04	0.27	0.03	0.0538	0.0367	0.9582
F. verticillioides	0.9129	0.0045	0.7563	0.57	0.55	0.72	0.99	0.11	0.4641	0.0016	0.0295
M. nivale	0.2647	< 0.0001	0.9349	1.24	1.01	3.70	0.82	0.11	< 0.0001	0.0031	< 0.0001
Fusarium spp.[e]	0.6324	0.0002	0.9086	0.28	0.34	0.89	0.15	0.04	0.0017	0.1098	0.0001

[a]Least square means estimates.
[b]Cultivar (C) by Zone (Z) interaction.
[c]Pairwise contrasts between regional zones (C = Central, N = North, S = South).
[d]Model did not converge. Seed infection incidences given here are arithmetic means.
[e]*Fusarium* species that were rare or not identified to species.

also seen in the longer-term climatological series. Trends in the maximum temperatures were not as obvious, but the central zone appeared to have had lower minimum temperatures during the flowering period in 1999–2002 when compared with the other zones (Table 5).

Discussion

As FHB is caused by several species of *Fusarium*, it is critical to identify the predominant species involved in order to prioritize research needs on other aspects of this disease. The species profile may vary temporally, regionally, or by cultivar, which presents a level of difficulty in designing, conducting and interpreting surveys on the species associated with FHB. In this study we looked for general trends in seed infection by *Fusarium* species in Italian wheat production over a 4 year period. We found that the main species associated with seed infection in this time frame were *F. graminearum*, *F. poae* and *M. nivale*. The durum cultivars in the study appeared to be more susceptible to seed infection by *Fusarium* species. Moreover, within Italy there was a pronounced geographical difference in the risk of seed infection by these pathogens.

Our study complements, and expands on, previous studies of FHB in Italian wheat. In those studies, the species most commonly associated with FHB in soft and hard wheat in Italy were *F. graminearum*, *F. culmorum* and *F. avenaceum* (Pancaldi and Torricelli, 1999), although *F. poae* could be isolated from soft wheats (Balmas and Corazza, 1994). In durum wheat, *F. graminearum* and *F. culmorum* predominated in FHB-affected spikes (Balmas et al., 1999).

The general findings of regional and wheat type differences were also evident in earlier surveys, although they are more limited in scope and inference. For example, Pasquini et al. (2001) reported that the incidence of FHB was highest in the central regions, but absent in the south. *Fusarium graminearum* was more prevalent in the north (Balmas et al., 1999). Balmas et al. (1999) also indicated that hard wheats were more susceptible to FHB than soft wheats.

While there were commonalities in the present and past survey work (such as the finding that *F. graminearum* was more prevalent in the north), there were also some notable differences. In contrast to the 2000 survey done by Pasquini et al. (2001), in that same year in our soft wheat plots the mean incidence of seed infection by *F. graminearum* was highest in the north (mean 14.3%;

Table 4. Mean seed infection incidence by *Fusarium* species for durum wheat cultivars grown in three regional zones (north, central, south) in Italy, 1999–2002. Contrasts between the cultivars as well as the regional zones are shown

Pathogen	p value			Incidence of seed infection (%) by cultivar			Contrast p value[b]			Incidence of seed infection (%) by regional zone			Contrast p value[b]		
	Cultivar	Zone	C*Z[a]	Appulo	Simeto	Zenit	App-Sim	App-Zen	Sim-Zen	North	Central	South	C–N	C–S	N–S
F. avenaceum	< 0.0001	< 0.0001	0.0002	0.69	1.21	0.37	.[c]	.	.	1.58	0.95	0.02	.	.	.
F. culmorum	0.1018	0.0470	0.4145	0.21	0.37	0.14	0.1592	0.4890	0.0371	0.34	0.35	0.05	0.9364	0.0257	0.0515
F. equiseti	0.0164	0.0042	0.0018	0.36	0.51	0.28	.[c]	.	.	0.87	0.36	0.03	.	.	.
F. graminearum	0.0045	< 0.0001	0.0235	1.14	1.88	1.60	0.0011	0.0312	0.2290	4.69	1.45	0.13	0.0021	0.0025	< 0.0001
F. poae	< 0.0001	0.0033	0.7836	0.93	2.68	2.04	< 0.0001	0.0007	0.1113	2.33	2.68	0.77	0.6557	0.0013	0.0111
F. proliferatum	0.3083	0.2365	0.2690	0.07	0.19	0.11	0.1311	0.5774	0.3393	0.06	0.26	0.05	0.1661	0.1292	0.9451
F. semitectum	0.0157	0.0023	0.1032	0.74	0.82	0.43	0.5612	0.0267	0.0057	1.15	0.91	0.09	0.5543	0.0039	0.0019
F. subglutinans	0.4500	0.1696	0.0812	0.21	0.34	0.25	0.2139	0.6454	0.4306	0.14	0.53	0.15	0.1137	0.1036	0.9690
F. verticillioides	0.0589	0.0071	0.6706	0.62	1.14	1.04	0.0252	0.0611	0.6964	1.19	1.48	0.30	0.5652	0.0027	0.0248
M. nivale	0.0211	< 0.0001	0.1200	1.49	1.76	2.22	0.2846	0.0065	0.0836	5.17	2.21	0.09	0.0083	< 0.0001	< 0.0001
Fusarium spp.[d]	0.0003	0.0016	0.4468	0.37	0.77	0.20	0.0080	0.1926	0.0001	0.50	0.77	0.09	0.2537	0.0005	0.0166

[a]Cultivar (C) by Zone (Z) interaction.
[b]Pairwise contrasts between the cultivars (App = Appulo, Sim = Simeto, Zen = Zenit) and regional zones (C = Central, N = North, S = South).
[c]Pairwise contrasts not done because of the significant ($p < 0.01$) C by Z interaction.
[d]*Fusarium* species that were rare or not identified to species.

Table 5. Weather during the mean flowering and early grain fill period in three regional zones in Italy, 1999–2002. Data were recorded at representative weather stations in each zone

Location[a]	1999	2000	2001	2002	Historical[b]
Durum wheat					
Rainfall (mm)					
Lodi (N)	7.7	65.6	52.5	87.9	29.8
Arezzo (C)	33.0	20.4	10.6	56.8	25.4
Foggia (S)	7.0	7.0	59.2	19.0	12.8
Maximum temperature (°C)					
Lodi (N)	23.0	24.1	23.2	18.6	20.1
Arezzo (C)	24.4	24.0	20.5	20.7	20.8
Foggia (S)	18.0	26.0	18.4	22.7	18.9
Minimum temperature (°C)					
Lodi (N)	12.4	13.1	12.4	10.1	9.4
Arezzo (C)	8.6	8.4	3.1	8.5	7.9
Foggia (S)	7.5	11.8	8.2	10.1	5.9
Soft wheat					
Rainfall (mm)					
Lodi (N)	7.7	65.6	52.5	87.9	29.8
Arezzo (C)	9.4	20.4	49.2	56.8	25.4
Foggia (S)	3.0	7.0	3.0	19.0	6.1
Maximum temperature (°C)					
Lodi (N)	23.0	24.1	23.2	18.6	20.1
Arezzo (C)	23.6	24.0	22.0	20.7	20.8
Foggia (S)	26.2	26.0	23.6	22.7	20.0
Minimum temperature (°C)					
Lodi (N)	12.4	13.1	12.4	10.1	9.4
Arezzo (C)	11.5	8.4	8.7	8.5	7.9
Foggia (S)	11.9	11.8	10.2	10.1	6.5

[a]Data recorded at weather stations (Lodi, Arezzo and Foggia) representative of the three regional zones (N = North, C = Center, S = South).
[b]Historical averages are from 1970–1998 for Lodi and Foggia, and from 1982–1998 for Arezzo.

range 1–34%), reduced in the central zone (2.65%; range 0–10%), but not completely absent from the south (0.38%; range 0–2%). The apparent differences between the studies are difficult to comment on without invoking unfounded speculation. They are impacted by different methodologies (freezing blotter versus plating of whole spikes on PDA), experimental locations, and time periods (the Pancaldi and Torricelli (1999) survey was from 1994–1996, and only covered the Emilia-Romagna region).

Our study demonstrated a consistent, increased risk of seed infection for the three durum CVs Appulo, Simeto and Zenit compared with the soft CVs Centauro and Eridano for all recovered species. Previous studies indicate that susceptibility is probably higher in durum cultivars than in bread wheat cultivars. For example, high incidence of FHB was reported in durum (Dill-Macky and Jones, 2000). In a cultivar trial, Southwell et al. (2003) found that the durum CVs Wollaroi and Yallaroi were more susceptible than the bread wheats.

Among the durum cultivars, our finding of differences among the cultivars in terms of seed infection by some species is also consistent with other reports. We also found that in the durum cultivars *F. graminearum* was one of the dominant species in the seed, which is consistent with other surveys of FHB in durum wheat (Lori et al., 2003; Southwell et al., 2003). Cultivar differences among durum wheats in the field were reported by Lori et al. (2003), though they postulated that some of the observed differences may have been due to the associated weather at the different heading times among cultivars. Lori and Sisterna (2001) examined durum seed collected from variety trials in the period 1995–1997, and found that there were differences among cultivars, years and locations. Differences among years and individual experimental locations are expected, because of weather. In the statistical model we explicitly accounted for the variation due to those factors, which allowed for more precise tests of the effects of wheat type, cultivar and zone.

The relative frequencies of *Fusarium* species on wheat spikes (or in seeds) can vary noticeably on a continental scale. Backhouse and Burgess (2002) showed that the general climate can restrict the range of species, or the predisposition to prevalence by *F. graminearum* among the species associated with FHB. In general, *F. graminearum* is the predominant species in warmer climates, such as in central and southern Europe (Parry et al., 1995). Other species, such as *F. avenaceum* and *F. culmorum* tend to assume greater importance in cooler, maritime climates. This appears to be the situation in Norway; a recent survey showed that the dominant species on wheat seeds were *F. avenaceum*, *F. poae* and *F. culmorum* (Kosiak et al., 2003).

There can be variations in the FHB species composition at a more regional scale, as evident in this study across Italian zones. Several other studies have reported on more localized, regional differences in FHB incidence. For example, Love and Seitz (1987) found that levels of infection by *F. graminearum* were higher in eastern parts of Kansas than in other parts of the state, an observation later supported by the work of Trigo-Stockli et al. (1998). In Argentina, the incidence of *F. graminearum* infection in durum wheat was found to decrease from eastern to western locations, paralleling the decrease in rainfall along that direction (Lori et al., 2003). We also observed the same general phenomenon in Italian wheat production, with the incidence of seed infection decreasing from the north to south in correlation with rainfall during the flowering period.

Species composition and relative frequencies of the pathogens within the FHB complex fluctuate not only in response to seasonal variation in environmental conduciveness, but may experience more permanent shifts. This seems to have happened in some parts of Europe and is in process in Canada. Waalwijk et al. (2003) showed that there has been a shift from *F. culmorum* to *F. graminearum* in the Netherlands. It appears that *F. graminearum* displaced *F. avenaceum* as the dominant pathogen in the FHB complex as it spread across Canada westwards (Clear and Patrick, 2000), thus changing the spectrum of the FHB complex in Canadian wheat production.

In this paper, we took a statistical approach to survey data, which not only identified the relative frequencies of the pathogens of the FHB complex in wheat seed, but also allowed for the quantification of the relative risks of seed infection across wheat types and production zones within Italy. Our results have identified the main *Fusarium* species involved in seed infection in Italy, and can be used to focus research efforts into other aspects of the disease. There nevertheless remains the need for continued surveys of the species associated with FHB; the shift in the species complex in Canada was revealed through the extensive survey work done by the Canadian Grain Commission on an annual basis in the 1990s.

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