Colonization of the Residues of Diverse Plant Species by *Gibberella zeae* and Their Contribution to Fusarium Head Blight Inoculum

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ABSTRACT

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The presence of *Fusarium* spp. was examined in the residues of wheat, barley, corn, sunflower, pasture, and gramineous weed species common in wheat and barley cropping systems collected from no-tillage and reduced-tillage plots from February 2001 to March 2003 in Uruguay. *Gibberella zeae* was recovered from residues of wheat, barley, corn, sunflower, fescue, and the gramineous weeds *Digitaria sanguinalis*, *Setaria* spp., *Lolium multiflorum*, and *Cynodon dactylon*, except from birdsfoot trefoil or white clover. Of the *Fusarium* spp. obtained, *G. zeae* was the most frequently recovered from wheat and barley residues, while other species were more common in other crops. *G. zeae* declined over time in all residues examined. Wheat and barley residues produced more ascospores of *G. zeae* than corn or other gramineous residues. Sunflower residue did not support ascospore production, indicating that it probably did not contribute to primary inoculum. Wheat and barley residues supported *G. zeae* colonization longer in no-till than in reduced-tillage production systems and, thus, may represent major contributors to Fusarium head blight (FHB) inoculum in Uruguay. The presence of *G. zeae* in the gramineous components of pastures, weed species, and sunflower should be considered when implementing control strategies for FHB.

Fusarium head blight (FHB) is a devastating disease of wheat and barley in the southern cone of South America. FHB represents one of the main constraints for wheat and barley production in Uruguay, where moderate to severe outbreaks have occurred in 1 of every 2 years over the past decade (9,30,31). Epidemics have caused extensive damage through direct losses in grain yield and quality, particularly because of the presence of mycotoxins such as deoxynivalenol (DON) in harvested grain.

The main pathogen associated with FHB in Uruguay is *Gibberella zeae* (Schwein.) Petch (anamorph, *Fusarium graminearum* Schwabe) (2,3,33,35). *G. zeae* is capable of surviving saprophytically on host residues including wheat, barley, corn, sorghum, and oat; grasses of the genera *Agropyron*, *Agrostis*, *Andropogon*, *Botriochloa*, *Bromus*, *Cenchrus*, *Cortaderia*, *Digitaria*, *Echinochloa*, *Lolium*, *Panicum*, *Paspalum*, *Pennisetum*, *Poa*, and *Setaria*; and numerous nongramineous species (1,8,11–13,15,32,36,40).

Wheat, barley, and corn residues have long been regarded as the major sources of primary inoculum (39,40,44) in FHB epidemics. The quantity of initial inoculum produced, principally ascospores (20,36), is reported to be directly related to the amount of colonized host residues at the soil surface (10,18,32). The importance of primary inoculum in FHB development, which is considered a monocyclic disease, justifies the use of cultural management practices, including rotation with nonhosts or the destruction of crop residues (16,17,22,43), for disease control.

The increased use of soil conservation practices in Uruguay since 1992 (21,23) and the diversity in pasture species, common weeds, and crops prevalent in mixed production systems (agriculture and livestock production) raise questions about the ecology of *G. zeae* and the epidemiology of FHB. Establishing effective strategies to manage *G. zeae*-infested residues requires an understanding of the contribution of these residues to the survival and production of *G. zeae* inoculum. Studies also are needed to identify agronomic practices that may aid in reducing the inoculum pressure from *G. zeae* in Uruguayan production systems.

The objectives of this study were to quantify *G. zeae* survival and inoculum production on a range of gramineous and nongramineous species common in Uruguayan production systems.

MATERIALS AND METHODS

Experimental site. Experimental plots were established in 1996 at a field site at

INIA La Estanzuela (INIA, National Institute for Agricultural Research, Uruguay) on a clay-loam soil (organic matter 2.1 to 4%; pH 6.2 to 7.0; Al < 0.1 meq/100 g; P > 5 ppm). Treatments consisted of combinations of crop rotation and tillage practices. The experiment was a randomized complete block design (RCBD) with three replicates. The two crop rotations examined were continuous agriculture (oats-corn/barley-sunflower/wheat) and a crop-pasture rotation (oats-corn/barley-sunflower/wheat/3 years of pasture). Pasture was a mixture comprising fescue (*Festuca arundinacea* L.), white clover (*Trifolium repens* L.), and birdsfoot trefoil (*Lotus corniculatus* L.). The tillage treatments were reduced tillage and no-till. Reduced tillage consisted of vertical tillage with a chisel plow or eccentric disc. No-till plots were not cultivated and were seeded with a direct drill (model 750; Deere and Company, Moline, IL).

Oat crops were planted on 11 April 2001 and 26 April 2002 and grazed by sheep to ground level at stem elongation (Zadoks growth stage [GS] 31 to 32; 46) on 10 July 2001 and 21 July 2002, leaving almost no residue. The other winter crops (wheat and barley) were planted on 6 July 1999, 30 June 2000, 4 July 2001, and 22 June 2002 and harvested at maturity (16 December 1999, 13 December 2000, 26 and 27 December 2001, and 11 December 2002). Plots of corn, a summer crop in Uruguay, were planted on 17 October 1996, 5 October 1997, 23 October 1998, 7 October 1999, and 25 October 2001 and harvested on 3 April 1997, 12 March 1998, 7 April 1999, 1 April 2000, and 5 April 2002. Similarly, sunflower (a summer crop) plots were planted on 20 December 2000 and 30 December 2001 and harvested on 10 April 2001 and 24 April 2002. Pasture was sown on 12 June 2002. For analysis, residue was arbitrarily categorized into two categories by age: category 1, residue 365 or fewer days old and category 2, residue 366 or more days old.

Plots were treated with the appropriate herbicides to control weeds. Soil samples (0 to 15 cm in depth) were collected from each plot prior to the planting of each crop and soil fertility was analyzed. Fertilizer was applied in accordance with standard agricultural practices in Uruguay (28).

Residue sampling and determination of residue decomposition and survival of *G. zeae* and other *Fusarium* spp. Residue was sampled every 3 months from Feb-

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ruary 2001 until March 2003 to estimate the amount of each type of residue on the soil surface and the colonization of residues by *G. zeae*. All visible residues at the soil surface were collected from five arbitrarily selected quadrats (0.5 by 0.5 m) in each plot. The residue collected from each quadrat was air dried at 25 to 30°C for 24 h. Residue was separated visually by crop and then the weight of residue from each previous crop was determined. Samples were stored at -10°C until processed.

Every 6 months from February 2001 until January 2003, *Fusarium* spp. other than *G. zeae* present on the collected residues were identified and the potential for inoculum production by *G. zeae* was assessed. Determination of *G. zeae* colonization was performed on 1.5- to 2-cm-long stem pieces, including a node in the case of gramineous species. A maximum of 30 pieces was assessed from each residue species per sample. However, where fewer than 30 pieces were recovered, all pieces recovered were assessed. Residue pieces were surface disinfected by being immersed in 0.5% sodium hypochlorite for 30 s, immersed in 70% ethanol for 15 s, rinsed three times in sterile deionized water, and blotted dry on sterile filter paper. Surface-disinfected residue pieces then were placed on pentachloronitrobenzene (PCNB) agar medium (15 g of peptone, 1 g of KH_2PO_4 , 0.5 g of MgSO_4 , 1 g of Terachlor [75% PCNB wt/wt], 15 g of agar, 0.24 g of neomycin sulfate, and 1 g of streptomycin sulfate per liter of deionized water) (25) in 9-cm-diameter plastic petri dishes (10 nodes per plate, three plates per sample). Plated residue pieces then were incubated for 7 days at 20 to 22°C with 12-h light and dark cycles provided by a 1:1 combination of cool-white (F40/CW Worklite 25; Phillips, Somerset, NJ) and blacklight (F40T12/BLB TL40W/08; Phillips) fluorescent lights. Colonies growing with salmon to pink-white mycelium were recorded as *Fusarium* spp. *G. zeae* colonies were estimated by transferring 10 arbitrarily selected colonies (50 colonies per treatment) identified as *Fusarium* spp. to carnation-leaf piece agar (CLA) medium (three to five sterile carnation leaf pieces [5 to 7 mm in diameter] per 2 ml of 1.5% water agar) (14) and potato-dextrose agar (PDA). Cultures on CLA and PDA were incubated at 20 to 22°C with 12-h light and dark cycles for 10 days. Bluish to black perithecia formation on CLA cultures confirmed the presence of *G. zeae* isolates. *Fusarium* colonies not forming perithecia were identified to species using the procedures outlined by Nelson et al. (27) and Burgess et al. (5).

Ascospore production. Ascospore production by *G. zeae* was determined on a known weight of residue that comprised node pieces for wheat, barley, corn, and gramineous weed residues, stem pieces for sunflower residue, and pieces of cobs for

corn residue. Residue pieces were surface disinfested as described previously and placed on the surface of sterile sand (150 g) moistened with sterile deionized water (20 g) within plastic boxes (200 by 300 by 70 mm, model Maxi N.1; Plasútil, São Paulo, Brazil). To facilitate perithecia development, the boxes containing the residue pieces were incubated at 20 to 22°C under 12-h light and dark cycles for 21 days. Deionized water was added as needed based on the gross container weight to maintain moisture within the boxes. Following the incubation period, the residue pieces were placed in a solution of sterile deionized water and polyoxyethylene 20-sorbitan monolaurate (Tween 20; Fischer Scientific, Fair Lawn, NJ). The dilution ratio used was 1 g of residue to 20 ml of sterile water to 0.1 ml of Tween 20. The residue pieces were left in solution for 12 h to allow ascospore discharge and then vigorously shaken for 10 min. Three 1-ml aliquots of the wash solution were obtained from each treatment and ascospore concentration was determined by counting ascospores in a known volume (0.02 ml) of the suspension in each sample. Spores were counted under a compound microscope and expressed as the number of ascospores per gram of residue. The number of ascospores per square meter was calculated based on the number of ascospores produced per gram of residue and the amount of each residue type per square meter, expressed as a mean of five quadrats.

Disease incidence and severity. The incidence and severity of FHB in wheat and barley plots were assessed on wheat and barley plants at mid- to late-milk stage (Zadoks GS 75-77; 46) in 2001 and 2002. A hundred spikes were assessed in each plot, consisting of 10 primary heads at each of 10 arbitrarily selected sites within the center rows of each plot. FHB incidence was determined as the percentage of spikes with symptomatic spikelets. FHB severity was determined as the percentage of symptomatic spikelets in all 100 spikes. In both years, wheat cv. INIA Cabure, which is moderately resistant to moderately susceptible to FHB (6), was planted. Susceptible barley cv. Estanzuela Quebracho was planted in 2001 and moderately susceptible cv. INIA Ceibo was planted in 2002 (6).

Pathogenicity tests. Pathogenicity tests were conducted with 14 arbitrarily selected *Fusarium* isolates recovered from residues collected in either November 2002 or January 2003. The isolates comprised *F. equiseti* recovered from *Digitaria sanguinalis* collected in November 2001, *F. sambucinum* isolated from the 2001 barley residue, *F. avenaceum* recovered from the 2002 wheat residue, *F. poae* recovered from the 2002 barley residue, *F. subglutinans* from the 2001 corn residue, and *F. solani* from the 2001 sunflower residue. *F.*

graminearum isolates recovered from the 2001 and 2002 wheat residue also were tested, as well as isolates from the 1999 and 2001 corn residues, the 2001 and 2002 barley residues, the 2001 sunflower residue, and *D. sanguinalis* collected in November 2002. Tests to determine the ability of isolates to induce disease in wheat and barley spikes were conducted in the greenhouse on plants of the susceptible wheat line LE 2294 and the susceptible barley cv. Estanzuela Quebracho. Seed were sown in plastic pots (150 by 150 mm in diameter by height) containing a 1:1:1 mixture of sand:soil:composted manure (Plantmax, Eucatex, Brazil). Four to five seeds were planted per pot and thinned to two plants when seedlings were at the three-leaf stage (Zadoks GS 13; 46). Plants were maintained at 20 to 25°C with alternating 12-h light (high-pressure sodium SON lamps, 400W; Phillips, Belgium) and dark periods. Plants were fertilized every 2 weeks with soluble N-P-K (N-P-K 12-8-5 + micronutrients; ISUSA foliar fertilizer, San José, Uruguay) applied at 30 ml per pot of a solution with a concentration of 0.4 liter/liter.

Monoconidial isolates were tested for pathogenicity. Conidial inoculum was produced on soybean medium (40 g of soybeans boiled in approximately 900 ml of deionized water for 25 min, filtered through two layers of cheesecloth, and adjusted to 1 liter with additional deionized water; and 15 g of agar) in petri plates incubated at 20 to 22°C with 12-h light and dark cycles for 14 days. Conidia were harvested by adding 10 to 15 ml of sterile distilled water to each plate and gently loosening spores with a sterile cover glass. Inoculum concentrations were adjusted to 5×10^4 conidia/ml. One to two spikes per pot (five pots per isolate per plant species) were inoculated at mid-anthesis (Zadoks GS 65; 46) with an airbrush (model VL3; Paasche Air Brush Company, Har Wood Heights, IL) operated at 0.84 kg cm⁻². Wheat and barley plants sprayed with sterile deionized water were included as controls. Plants were incubated in a dew chamber at 20 to 22°C with a 12-h photoperiod and 100% relative humidity for 72 h. After incubation, plants were returned to the light and temperature conditions used prior to inoculation. Disease severity was evaluated 21 days after inoculation and expressed as the percentage of symptomatic spikelets per spike.

Statistical analyses. Survival of *G. zeae* and ascospore production did not meet assumptions of normality with binomial and Poisson distributions, respectively. Data were analyzed using generalized linear models (26) (SAS procedure GENMOD, version 8.1; SAS Institute Inc., Cary, NC). Tests of significance were based on likelihood ratio statistics.

Residue dry matter, FHB severity, FHB incidence, and pathogenicity data were

subjected to analysis of variance using the GLM procedure of SAS (version 8.1; SAS Institute Inc.). Where the *F* ratio was significant (*P* < 0.05), differences among treatment means were determined using protected least significant differences (LSD).

RESULTS

Average mean temperatures in 2001 during summer (January, February, March), autumn (April, May, June), winter (July, August, September), and spring (October, November, December) were 23, 14, 13, and 19°C, respectively. In 2002, mean temperatures during these seasons were 27, 18, 17, and 25°C, respectively. Average mean temperature during summer 2003 was 22°C.

Total rainfall during 2001 was 1,513 mm, with rainfall of 549 mm (30 rain events) in summer, 191 mm (23 rain events) in fall, 230 mm (25 rain events) in winter, and 543 mm (36 rain events) in spring. In 2002, total rainfall was 1,222 mm, with 360 mm (32 rain events) in summer, 291 mm (21 rain events) in fall, 212 mm (19 rain events) in winter, and 359 mm (26 rain events) in spring. Total rainfall in summer 2003 was 342 mm (23 rain events).

Residue decomposition. Significant reductions over time in the dry weight of residues were found in all treatments over the 25 months when samples were recovered (Table 1). No-till treatments had more residues compared with reduced-tillage treatments (Table 1). Significant differences were found for individual sampling dates between residue dry weight of no-till and reduced-tillage treatments, except for sampling dates when residue was sampled following harvest but before tillage operations were implemented (Table 1).

No-till corn produced more weight of residue than the other crops examined. Gramineous weeds generated less residue than the other residues recovered. The weights of both cereal and sunflower residues older than 1 year were significantly reduced compared with residues recovered when less than 1 year old (Table 1).

Survival of *G. zeae* on residues. *G. zeae* was isolated from all the recovered residues of gramineous species, regardless of treatment or sampling time. The relative level of *G. zeae* recovered from all cereal crop residues decreased with residue age (Fig. 1). *G. zeae* was isolated from corn residues up to 3 years after harvest, though residues were recovered until the fourth

year after harvest. Based on the percentage of residue pieces colonized, wheat and barley residues had higher levels of *G. zeae* colonization than the residues of corn, gramineous weeds (*D. sanguinalis*, *Cynodon dactylon*, *Lolium multiflorum*, and *Setaria* spp.), fescue, or sunflower (Fig. 2). No colonization of forage legumes by *G. zeae* was detected.

Sunflower residue was found to be a substrate for the survival of *G. zeae*. Although *G. zeae* colonized sunflower residue, *G. zeae* was recovered at lower levels from this residue than from the cereal crop residues examined. Furthermore, *G. zeae* was recovered only from sunflower residues up to 1 year old (Fig. 1).

Barley and gramineous weed residues recovered from no-till plots were more highly colonized by *G. zeae* than residues recovered from reduced-tillage plots (Fig. 2). No significant differences were found among tillage treatments for the other residues examined, except at occasional sampling dates (i.e., corn residue in February 2001 and wheat residues in October 2001 and January 2003).

G. zeae was the *Fusarium* sp. recovered most frequently from wheat and barley residues sampled in the first 10 to 12

Table 1. Dry weight of seven types of residues (wheat, barley, corn, sunflower, fescue, forage legumes, and gramineous weeds) recovered from two tillage systems

System ^y	Dry weight (g/m ²) on sampling date ^x										Mean ^z
	2001					2002					
	Feb	Apr	Jul	Oct	Dec	Mar	Aug	Nov	Jan	Mar	
Wheat											
NT1	350 b	115 b	105 c	41 cd	146 b	81 b	35 de	19 d	110 a	84 bc	121 b
NT2	12 d	nrr	nrr	nrr	56 d	21 d	nrr	nrr	3 b	nrr	26 def
RT1	372 ab	26 cd	6 ef	3 e	139 b	21 de	9 ef	34 cd	136 a	16 fg	84 c
RT2	nrr	nrr	nrr	nrr	12 e	18 de	nrr	nrr	6 b	nrr	14 ef
Barley											
NT1	400 a	181 a	117 bc	68 bc	238 a	103 ab	18 ef	8 d	100 a	79 bc	131 b
NT2	nrr	nrr	nrr	nrr	23 de	nrr	nrr	nrr	nrr	1 g	12 ef
RT1	323 b	43 c	18 de	5 e	113 c	48 c	6 d	nrr	108 a	38 defg	77 c
RT2	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	...
Corn											
NT1	339 b	nrr	nrr	nrr	nrr	nrr	176 a	228 a	132 a	130 a	201 a
NT2	18 d	42 c	29 d	6 e	7 e	5 de	3 f	1 d	nrr	nrr	15 ef
RT1	174 c	nrr	nrr	nrr	nrr	nrr	118 b	254 a	18 b	15 fg	116 b
RT2	4 d	2 c	2 ef	10 de	7 e	nrr	7 f	nrr	nrr	11 fg	5 f
Sunflower											
NT1	nrr	167 a	162 a	209 a	166 b	115 a	180 a	88 bc	24 b	27 efg	123 b
NT2	nrr	nrr	nrr	nrr	nrr	nrr	63 d	19 d	8 b	2 g	23 def
RT1	nrr	183 a	133 b	72 b	48 d	11 de	93 c	106 b	17 b	20 efg	76 c
RT2	nrr	nrr	nrr	nrr	nrr	nrr	30 e	9 d	4 b	3 g	11 ef
Fescue											
NT	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	40 cdef	40 cdef
RT	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	53 bcde	53 cde
Forage legumes											
NT	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	85 b	85 bc
RT	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	nrr	68 bcd	68 bc
Gramineous weeds											
NT	1 d	17 cd	1 f	1 e	20 e	14 de	1 f	nrr	2 b	20 fg	8 ef
RT	1 d	nrr	nrr	nrr	8 e	4 e	nrr	nrr	1 b	14 fg	4 f

^x Values given are mean dry weight of all residue pieces sampled for a given treatment. Values with different letters in the same column are significantly different at *P* = 0.05 according to least significant difference tests. Bold text indicates residue from recently harvested crops; nrr = no residues recovered, either because residue was fully decomposed or because residues were not yet present in the cropping sequence.

^y Tillage system and residue age. NT = no till and RT = reduced tillage. Residue was collected approximately every 3 months from February 2001 to March 2003 and divided according to age (1 = 365 or fewer days old and 2 = older than 366 days).

^z Mean of all sampling dates.

months after harvest. Recovery of other species of *Fusarium* increased over time. *F. equiseti*, *F. oxysporum*, and *F. solani* were recovered in all five plant residues examined. *F. avenaceum* and *F. sambucinum* were recovered exclusively from wheat, barley, and gramineous weeds residues. *F. acuminatum* and *F. poae* were recovered from wheat, barley, gramineous weeds, and sunflower residues. *F. tricinctum* was recovered only from wheat and

barley residues, while *F. semitectum* was recovered from barley and sunflower residues. *F. subglutinans* was recovered from the residues of barley, corn, and gramineous weeds. Other *Fusarium* spp. recovered included *F. culmorum* from wheat residues and *F. verticillioides* from corn residues.

Ascospore production. Wheat and barley residues produced more *G. zeae* ascospores per gram of residue, whereas gramineous weeds produced fewer asco-

spores compared with the other residues examined (Table 2). Although the production of ascospores was at low levels, gramineous weeds supported year-round ascospore production. Even though *G. zeae* colonized sunflower residue, it did not produce ascospores on this substrate.

Inoculum production per unit area, calculated using ascospore production and residue coverage data, was greater in 1-year-old and younger residues compared

Fig. 1. *Gibberella zeae* colonization of five different residues (wheat, barley, corn, sunflower, and gramineous weeds [*Digitaria sanguinalis*, *Cynodon dactylon*, *Lolium multiflorum*, and *Setaria* spp.]) recovered from two tillage systems: no tillage (solid line) and reduced tillage (dashed line) in residues sampled from February 2001 to March 2003. Each point represents the mean percentage of all colonized residue sampled. Vertical bars represent standard errors.

with older residues (Table 3). When comparisons were performed among cereal residues, winter cereal residues produced more ascospores per square meter ($2,442 \times 10^3$) over the entire sampling period than

did corn residue (328×10^3 ; data not shown). However, for some sampling dates, such as February 2001 and August 2002, no significant differences were found for ascospore production per unit

area between winter cereal residues and corn residues (Table 3). When sampling for residue was carried out immediately after winter cereal harvest and prior to tillage operations (December, January, or February), there were no significant differences in the number of ascospores produced per square meter between no-till and reduced-tillage treatments.

Disease incidence and severity. In wheat, the incidence of FHB was higher in 2001 than 2002. In 2002, no-till wheat had higher FHB incidence than wheat under reduced tillage. The incidence of FHB in barley in both 2001 and 2002 was over 94%, and no significant effects of tillage treatment were observed. The incidence of FHB in wheat and barley was influenced by year (Table 4).

Disease severity also was greater in both wheat and barley in 2001 than in 2002 (Table 4). Although FHB was less severe in 2002, the severity in no-till plots was greater than in the reduced-tillage treatments in both crops. In both 2001 and 2002, the severity of FHB was greater in barley than wheat (Table 4).

Pathogenicity tests. All isolates tested caused symptoms of FHB on wheat and barley spikes, except when wheat was inoculated with *F. subglutinans* (Table 5). In both wheat and barley, the effect of the isolate on FHB severity was significant. Spikes, mock-inoculated with sterile water to serve as a control treatment, did not show any symptoms of FHB. The mean FHB severity of all isolates tested was lower in wheat than the mean FHB severity for inoculated barley plants (Table 5).

The *G. zeae* isolates from 2002 wheat residue produced the greatest disease severity on both wheat and barley plants. On wheat plants, *G. zeae* isolates caused significantly greater disease severity compared with all other isolates tested except the *F. avenaceum* isolate. In barley, the isolate of *G. zeae* from 2002 wheat residue also caused the highest FHB severity of the isolates tested, but not significantly more than the isolate of *G. zeae* from 2002 barley residue or the *F. equiseti* and *F. sambucinum* isolates tested.

DISCUSSION

Results from this study show that *G. zeae* can survive and produce inoculum on wheat, barley, corn, and gramineous pasture or weed species, indicating that multiple residue types can be long-term sources of *G. zeae* inoculum. These findings agree with earlier studies in other wheat and barley production areas (11–13,36,40).

Our findings suggest that birdsfoot trefoil and white clover, which are common components of perennial pastures in Uruguay, do not appear to support *G. zeae* or contribute to inoculum. Fernandez (12) was unable to recover *G. zeae* from naturally infected fields of birdsfoot trefoil, only recovering the fungus from artificially

Fig. 2. *Gibberella zeae* colonization of six different residue types (wheat, barley, corn, sunflower, fescue [Fes; *Festuca arundinacea*], and gramineous weeds [G.w.; *Digitaria sanguinalis*, *Cynodon dactylon*, *Lolium multiflorum*, and *Setaria* spp.]) recovered from February 2001 to March 2003 from two tillage systems (NT = no tillage and RT = reduced tillage). Residue was divided by age (1 = 365 or fewer days old and 2 = older than 365 days). Values presented are the mean percentage of all colonized residue sampled. Values with different letters are significantly different at $P = 0.05$ based on likelihood ratio statistics. Vertical bars represent standard errors.

Table 2. Ascospores of *Gibberella zeae* produced per gram of residue (wheat, barley, corn, sunflower, or gramineous weeds)

	<i>Gibberella zeae</i> ascospores (ascospores per gram of residue) ^y				
	2001				
System ^z	February	July	December	August 2002	January 2003
Wheat					
NT1	14,267 a	4,667 a	32,644 c	4,667 abc	14,933 a
NT2	5,334 bcd	nrr	6,667 d	nrr	nra
RT1	5,556 bc	nra	54,111 ab	12,333 ab	13,600 a
RT2	nrr	nrr	4,667 d	nrr	nra
Barley					
NT1	13,327 a	2,889 ab	58,333 b	14,333 a	8,400 b
NT2	nrr	nrr	nra	nrr	nrr
RT1	6,500 b	2,500 b	84,430 a	nra	17,867 a
RT2	nrr	nrr	nrr	nrr	nrr
Corn					
NT1	7,467 b	nrr	nrr	2,667 bc	5,067 b
NT2	2,667 cd	1,600 b	16,000 cd	1,000 c	nrr
RT1	1,667 d	nrr	nrr	3,333bc	3,733 bc
RT2	nra	nra	nra	nra	nrr
Sunflower					
NT1	nrr	0 c	0 e	0 d	0 d
NT2	nrr	nrr	nrr	0 d	0 d
RT1	nrr	0 c	0 e	0 d	0 d
RT2	nrr	nrr	nrr	0 d	0 d
Gramineous weeds					
NT	2,500 cd	nra	4,000 d	2,667 bc	1,867 c
RT	2,667 cd	nrr	nra	nrr	nra

^y Values with different letters in the same column are significantly different at $P = 0.05$ based on likelihood ratio statistics. Bold text indicates residue from recently harvested crops, nra = no residue available for analysis of ascospore production after the analysis of dry weight was completed, and nrr = no residue recovered.

^z Tillage system and age group of residue. Residues were collected from two tillage systems (NT = no till and RT = reduced tillage) from February 2001 to March 2003 and divided into two age groups (1 = 365 or fewer days old and 2 = older than 366 days).

inoculated plants. Other legumes commonly found in perennial pastures in Uruguay, such as alfalfa (*Medicago sativa* L.) and red clover (*T. pratense* L.), have been cited as hosts of *G. zeae* (12,43) but were not evaluated in this study.

Sunflower residue was able to support low levels of *G. zeae* colonization; however, the production of perithecia was not observed on this substrate. To our knowledge, this is the first report indicating that sunflower residue is colonized by *G. zeae*. However, *G. zeae* has been reported as part of the mycoflora present in sunflower florets (37). The fact that no perithecia production was observed in this residue might indicate that the conditions in this study were not optimal or that the biochemical composition of the residue did not support perithecia production.

Gramineous species that flower during summer, whether components of pastures (fescue) or weeds (*D. sanguinalis*, *C. dactylon*, and *Setaria* spp.), could contribute ascospore inoculum in Uruguayan no-till production systems during the winter and early spring when wheat flowering and barley heading occur. Even though fescue was not evaluated for perithecia or ascospore production during the course of this study, fescue has been observed to support perithecial production after ripening (31) and, therefore, also may contribute to the inoculum pressure in growers' fields during spring.

Corn residue was colonized at lower levels compared with winter cereal residues, yet the residue supported *G. zeae* for longer periods of time as a consequence of the slower decomposition of corn residue. Ascospore production per unit area in corn was lower than for wheat and barley residues over the entire sampling period. Corn is used mostly in rotations in dairy production systems in the southern part of southwestern Uruguay. Although corn is planted in rotation with wheat and pastures on these farms, it generally is used for silage and, consequently, much smaller amounts of residue are left in the field compared with field plots examined in this study. The low levels of ascospore production and limited residues left in fields indicate that the contribution of corn to *G. zeae* inoculum in Uruguayan production systems is probably low, in contrast to many parts of North America and Europe where corn is frequently cited as being a major factor contributing to FHB epidemics (10,19,38,39,41).

The decline in the colonization of residues by *G. zeae* and inoculum production over the 2 years of this study parallel residue decomposition and was expected based on previous studies examining *G. zeae*-infested wheat (18,32,45) and corn (18) residues over time. In this study, wheat and barley residues decomposed faster than corn residues, especially in the first few months after harvest. The rate of

residue decomposition depends on many factors, including the biochemical form of the residue and the suitability of the environment and the availability of nutrients, especially nitrogen, for the microbes involved in decomposition. The rapid decomposition of the wheat and barley might be due to the fact that the subsequent summer crops provided an environment that promoted microbial activity by increasing the moisture present in residues for long periods. The warm, moist conditions below

the canopy of summer crops, as well as reduced light, also might be detrimental to the production of perithecia by *G. zeae*. Corn residue decomposed more slowly than winter cereals, largely because low temperatures prevail after the harvesting of corn in April, whereas warmer temperatures follow the harvest of winter cereals in November to December. Different patterns of inoculum production in the different plant residues over time also might have reflected the differential nutritional status

Table 3. Number of ascospores ($\times 10^3$) of *Gibberella zeae* produced per square meter on wheat, barley, corn, sunflower, and gramineous weeds residues from two tillage systems

System ^z	<i>G. zeae</i> ascospores (ascospores per square meter of residue) ^y				
	2001			August 2002	January 2003
	February	July	December		
Wheat					
NT1	4,907 ab	486 a	3,570 b	163 ab	1,639 a
NT2	162 d	nrr	369 c	nrr	nra
RT1	2,074 b	nra	8,238 ab	113 b	1,844 a
RT2	nrr	nrr	52 d	nrr	nra
Barley					
NT1	5,397 a	400 a	13,871 a	264 ab	773 b
NT2	nrr	nrr	nrr	nrr	nrr
RT1	2,098 b	43 b	9,331 ab	nra	1,928 a
RT2	nrr	nrr	nrr	nrr	nrr
Corn					
NT1	2,221 ab	nrr	nrr	482 a	667 b
NT2	50 d	48 b	462 c	3 c	nrr
RT1	287 cd	nrr	nrr	393 a	66 c
RT2	nra	nra	nra	nra	nrr
Sunflower					
NT1	nrr	0 c	0 e	0 d	0 e
NT2	nrr	nrr	nrr	0 d	0 e
RT1	nrr	0 c	0 e	0 d	0 e
RT2	nrr	nrr	nrr	0 d	0 e
Gramineous weeds					
NT	2 e	nra	58 d	2 c	3 d
RT	3 e	nrr	nra	nrr	nra

^y Values with different letters in the same column are significantly different at $P = 0.05$ based on likelihood ratio statistics; nra = no residue available for analysis of ascospore production after the analysis of dry weight was completed and nrr = no residue recovered.

^z Tillage system and age group of residue. NT = no tillage and RT = reduced tillage. Residue was collected from February 2001 to March 2003 and divided into two age groups (1 = 365 or fewer days old and 2 = older than 366 days).

Table 4. Fusarium head blight (FHB) incidence (%) and severity (%) in wheat and barley in 2001 and 2002 for two tillage treatments (NT = no tillage and RT = reduced tillage)^z

Crop	Treatment	Year		Mean
		2001	2002	
FHB incidence				
Wheat	NT	93 a	64 a	79 c
	RT	91 a	32 b	62 d
Mean	...	92 C	48 D	...
Barley	NT	100 e	94 f	97 g
	RT	100 e	96 f	98 h
Mean	...	100 G	95 H	...
FHB severity				
Wheat	NT	19.6 j	9.0 j	14.3 m
	RT	18.4 j	4.5 k	9.9 n
Mean	...	19.0 M	6.8 N	...
Barley	NT	54.8 p	21.9 q	38.4 s
	RT	51.6 p	18.2 r	34.9 t
Mean	...	53.2 S	20.1 T	...

^z Values given are the mean of five replicates. Values in a column, for either incidence or severity in either wheat or barley, followed by different lowercase letters are significantly different at $P = 0.05$ according to least significant difference (LSD) tests. Values in a row followed by different uppercase letters are significantly different at $P = 0.05$ according to LSD tests.

of each substrate, as suggested by Khonga and Sutton (18).

The rate of residue decomposition is inversely related to the amount of residue left on the soil surface (18,32). Contact of residue with soils, and especially the burial of residue, promotes greater microbial activity, accelerating decomposition (29). Residues in no-till systems decompose more slowly than residues under reduced-tillage systems; thus, residues in no-till systems likely would support the survival of *G. zeae* and inoculum production for longer periods of time.

When evaluating the effect of tillage systems on the incidence and severity of FHB in wheat and barley, we observed that disease development is highly dependent on climate. In years with extremely conducive climatic conditions from flowering to harvest for wheat and from heading to harvest for barley, such as occurred in 2001, tillage systems did not have a significant effect on FHB. In years with only moderately conducive conditions for FHB (i.e., La Estanzuela 2002), the impact of tillage on FHB development was apparent. Above-average rainfall, both amount and frequency, during spring 2001 undoubtedly contributed to the severe outbreak of FHB in commercial wheat and barley crops in Uruguay. Previous studies also suggest that weather conditions are more influential than tillage practices per se on the development of FHB (7,10,24).

G. zeae was the *Fusarium* sp. most frequently recovered from winter cereal residues in the first few months following harvest. *G. zeae* probably benefits in competition with other saprophytes from its ability to colonize wheat and barley residues as a parasite. Other *Fusarium* spp. known to have greater competitive saprophytic abilities in wheat or barley residues (i.e., *F. avenaceum* and *F. poae*) or in soil

(i.e., *F. oxysporum*) increased in prevalence as *G. zeae* populations decreased over time. These results support the findings from our previous study conducted in the Upper Midwest of the United States (32). In this study, species other than *G. zeae* dominated the *Fusarium* populations in nonwinter cereal residues. The most common species recovered were *F. verticillioides* in corn residues, *F. equiseti* in gramineous weeds residues, and *F. oxysporum* and *F. solani* in sunflower residues. *F. verticillioides* is known as a common pathogen of corn (42), while both *F. oxysporum* and *F. solani* have been reported as pathogens of sunflower (34). The dominance of pathogenic species in the residue of host plants supports the theory that species gain a competitive advantage as a saprophyte by establishing themselves as pathogens in host tissue (4).

The results of this study also confirm that isolates of *G. zeae*, regardless of the plant residue from which they were recovered, are capable of infecting wheat and barley plants. However, the aggressiveness of *Fusarium* isolates, as indicated by the relative severity of FHB in inoculated wheat and barley plants both within and among species, varies considerably. These findings are in agreement with recent studies examining the relative aggressiveness of *Fusarium* isolates (R. Dill-Mackay, unpublished data).

Based on the information generated in this study, we conclude that, under the mixed production systems of southwestern Uruguay, wheat and barley appear to be the most important species contributing to *G. zeae* inoculum. These residues should be avoided if wheat or barley would be planted, particularly under no-tillage systems. Priority should be given to planting wheat or barley in forage legume, sunflower, or perennial pasture residues. Even

when these last two options allow for the survival of *G. zeae* from one season to the next, they produce no primary inoculum (sunflower residue) or very low levels of inoculum (pasture).

Management of FHB is likely to involve a combination of resistance, use of fungicides, and cultural practices aimed at reducing the primary inoculum in wheat and barley crops. Practices such as tillage, residue management, and crop sequence play significant roles in determining the risk of FHB.

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Table 5. *Fusarium* head blight (FHB) severity 21 days after inoculation of wheat and barley plants with 14 isolates representing seven *Fusarium* spp. recovered from five crop residues collected between 1999 and 2002

<i>Fusarium</i> sp.	Isolates recovered from		FHB severity (%) ^z	
	Residue type	Year	Wheat	Barley
<i>Fusarium graminearum</i>	Wheat	2002	18.0 a	19.0 a
<i>F. graminearum</i>	Wheat	2001	8.2 b	4.3 cd
<i>F. graminearum</i>	Sunflower	2001	2.1 b	9.2 bcd
<i>F. graminearum</i>	Corn	2001	2.3 b	9.2 bcd
<i>F. graminearum</i>	Corn	1999	2.9 b	1.2 d
<i>F. graminearum</i>	Barley	2002	2.9 b	16.5 ab
<i>F. graminearum</i>	Barley	2001	3.7 b	2.2 d
<i>F. graminearum</i>	<i>Digitaria sanguinalis</i>	...	2.3 b	3.4 cd
<i>F. equiseti</i>	<i>D. sanguinalis</i>	...	5.1 b	16.4 ab
<i>F. sambucinum</i>	Barley	2001	0.6 b	12.5 abc
<i>F. avenaceum</i>	Wheat	2002	9.8 ab	6.4 cd
<i>F. poae</i>	Barley	2002	5.4 b	5.3 cd
<i>F. subglutinans</i>	Corn	2001	0 b	3.2 cd
<i>F. solani</i>	Sunflower	2001	2.7 b	2.0 d
Mean FHB severity (%)	...	...	4.7 B	7.9 A

^z Values given are the mean of 10 replicates. Values in a column followed by different lowercase letters are significantly different at $P = 0.05$ according to least significant difference LSD tests. Values in a row followed by different uppercase letters are significantly different at $P = 0.05$ according to LSD test.

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