



# Integrated Foliar Disease Management to Prevent Yield Loss in Argentinian Wheat Production

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## ABSTRACT

Zero tillage often leads to wheat (*Triticum aestivum* L.) yield losses from diseases caused by necrotrophic foliar pathogens. The aim of this work was to evaluate the combined effect of tillage, N fertilization, fungicides, and resistant cultivars in reducing foliar disease severity to prevent significant yield losses. A 2-yr study including combinations of (i) conventional and zero tillage; (ii) N fertilization rates 0, 80, or 160 kg ha<sup>-1</sup> N; (iii) two fungicide treatments (with and without a fungicide (1 L of metconazole, 9%) at growth stages (GS) 32 and 39; and (iv) three wheat cultivars was conducted in the Rolling Pampas region in Argentina. The most common foliar disease in the trial was tan spot [*Pyrenophora tritici-repentis* (Died.) Drechs.]. Conventional tillage reduced foliar disease severity at GS 23 by 46 and 56% and the area under disease progress curve (AUDPC) by 20 and 14% for each season, respectively compared with zero tillage. The cultivar Buck Bigua had significantly lower AUDPC values than the others. Fungicide and N application reduced disease severity at GS 23 by 35 and 34% respectively, on average over both years. Disease was less severe in zero tillage plots which received a fungicide compared to conventional tillage plots that were not treated with fungicide. In 2002 yields were greater in conventional tillage plots with 160 kg ha<sup>-1</sup> N and fungicide application than in all other treatments. In 2003 yields were greatest in zero tillage plots with 160 kg ha<sup>-1</sup> N and fungicide. The results of this study indicate that in spite of the increase of necrotrophic diseases, developing no-till systems in wheat monoculture is possible without significant yield losses if effective disease management practices are applied.

IN THE ROLLING Pampa region of Argentina, conservation management practices such as zero tillage are increasing as alternative cropping systems. Zero tillage systems have been implemented to restore soil structure in large areas cultivated with double-crop sequences such as wheat/soybean [*Glycine max* (L.) Merr.]; corn (*Zea mays* L.)–wheat/soybean; or wheat monoculture (Alvarez and Steinbach, 2009). Annual wheat/soybean double-crop sequences using conventional tillage are considered less desirable because of the effect on soil organic matter and the reduced quantity of residue that soybean crops leave after harvest (Fontanetto and Vivas, 1998). In the semiarid region of Argentina, conservation management techniques are also necessary to prevent soil erosion and effectively store and use the limited amount of precipitation for crop

production (Méndez and Buschiazzi, 2010). Zero tillage can also reduce costs by decreasing fuel consumption required to produce a crop (García et al., 2000).

However, in the wheat/soybean system under zero tillage, as in wheat following wheat, the inoculum of necrotrophic fungi usually survives until the next wheat season; typically, a minimum of 1 to 2 yr between wheat crops is required to reduce populations of these organisms (Duczek et al., 1999). In zero tillage systems, crop residue mineralization is slow. It requires 14 to 16 mo in Brazil (Reis and Carmona, 1995), but approximately 18 to 32 mo in Argentina and Uruguay due to lower average temperatures than in Brazil (Utermark, 1995; Cordo et al., 2005). Zero tillage may have a different effect on plant diseases depending on the soil type, geographic location, environment, and the biology of the particular disease-causing organism (Krupinsky et al., 2002). Previous studies reported that tan spot [*Pyrenophora tritici-repentis* (Died.) Drechs, anamorph *Drechslera tritici-repentis* (Died.) Shoemaker] and Stagonospora blotch [*Phaeosphaeria avenaria* (G.F. Weber) O. Eriksson f. sp. *triticea* T. Johnson, anamorph *Stagonospora avenae* (A. B. Frank) Bissett f. sp. *triticea* T. Johnson] increased in zero tillage systems in wheat monoculture or wheat following fallow, although the opposite occurred when wheat followed other crops (Bailey et al., 1995, 2000; Fernandez et al., 1998, 1999; Krupinsky et al., 2007b). Conventional tillage increases crop residue decomposition, reducing fungal inoculum (Sutton and Vyn, 1990; Fernandez et al., 1998). However, others (Bailey et al., 1995; Stover et al., 1996; Fernandez et

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**Abbreviations:** AUDPC, area under disease progress curve; IPAR, intercepted photosynthetic active radiation; KPS, kernels per spike; SPM2, spike per square meter; TKW, thousand kernel weight.

al., 1998; Bailey et al., 2000; Krupinsky and Tanaka, 2001; Krupinsky et al., 2002, 2004, 2007a) reported contrasting results regarding the effect of zero tillage on necrotrophic wheat diseases, depending on the environment and the crop growth stage evaluated (early or late in the season). Stover et al. (1996) and Krupinsky et al. (2004) reported that the greater early season foliar disease associated with chisel plowing did not consistently carry over to late season foliar ratings. Bailey et al. (1995, 2000) indicated that reduced tillage did not result in significant crop losses that require fungicide inputs to maintain crop yield or in economical important losses (Krupinsky et al., 2002). In addition, higher disease severities were associated with the no tillage treatment only with high precipitation levels (Krupinsky and Tanaka, 2001, Krupinsky et al., 2007a)

Fungicides are used to manage foliar wheat diseases in Argentina (Carmona et al., 1999a). The response to fungicide application depends on the severity of specific foliar diseases, cultivar disease resistance or tolerance, management practices, and environmental conditions (Roth and Marshall, 1987; Varga et al., 2005; Carignano et al., 2008). Jorgensen and Olsen (2007) reported wheat yield increases following fungicide treatments ranging from 0.8 to 4.4 Mg ha<sup>-1</sup>, depending on the amount of infested straw on the soil surface, disease severity, and fungicide strategy (type of active ingredient, timing or number of applications, rates and method of application). Increased yields are associated mainly with an increase in thousand kernel weight (TKW) (Gooding et al., 1994; Herrman et al., 1996; Puppala et al., 1998; Varga et al., 2005; Carignano et al., 2008), while other yield components such as spike per square meter (SPM2) (Varga et al., 2005) or kernels per spike (KPS) (Herrman et al., 1996; Kelley, 2001; Puppala et al., 1998; Varga et al., 2005) are usually not affected by disease severity. However, Simón et al. (2002) reported that preventing early wheat infection by *Septoria tritici* could result in an increase of SPM2 and KPS.

Management practices such as N fertilization can also affect the expression of wheat foliar diseases (Simón et al., 2002, 2003) and the effectiveness of foliar fungicide application (Howard et al., 1994; Simón et al., 2002, 2003; Varga et al., 2005). Increasing rates of N fertilizer may increase, decrease, or have no effect on foliar disease severity, depending on the geographic location (Krupinsky, 1999) and the type of disease. Increasing N fertilizer rates retarded tan spot development (Annone, 2004; Carignano et al., 2008; Huber et al., 1987; Krupinsky and Tanaka, 2001; Krupinsky et al., 2007a; Simón et al., 2004). However, Bockus and Davis (1993) suggested that N fertilizer applications do not directly affect tan spot severity, but rather appear to reduce disease impact through delayed leaf senescence. High N rates increase Septoria blotch or tan spot severity due to an increase in crop biomass production, which creates a micro-environment conducive to fungal development in humid regions (Cox et al., 1989; Howard et al., 1994; Roberts et al., 2004; Simón et al., 2002, 2003).

The major foliar fungal diseases caused by necrotrophic pathogens in Argentina have historically been tan spot and Septoria blotch, the latter caused by *Septoria tritici* Rob. ex Desm., teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn. (Annone, 1998, Carmona et al., 1999b). Together with some other pathogenic fungi (mainly *Bipolaris*

*sorokiniana* (Sacc.) Schoem., teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex Dastur and *Alternaria* spp.), tan spot and Septoria blotch form a leaf spot disease complex in Argentina. The proportion of each fungus in this complex may vary depending on the environment and geographic location (Perelló et al., 1996; Perelló and Moreno, 2004; Perelló and Sisterna, 2006). Conflicting reports regarding the effect of tillage practices on foliar plant diseases indicates that the impact of tillage systems in a particular region needs to be assessed through research (Sumner et al., 1981). Information regarding the incidence of foliar diseases as related to tillage, N fertilization, wheat genotype, pathogen genotype, and fungicide application practices is lacking. Although economic damage thresholds for fungicide applications to control tan spot in Argentina have been determined (Carmona et al., 1999a), how these threshold levels fit into an integrated management system has not been determined. We hypothesize that even with high inoculum pressure of common foliar pathogens under zero tillage practices, using resistant cultivars, optimizing N rates, and applying fungicide would reduce yield losses from disease. The main objective of this work was to examine the interaction among tillage systems, N fertilization rates, and fungicide applications on the wheat foliar disease complex, crop biomass, yield, and yield components in a wheat crop under continuous monoculture in the Pampa region of Argentina.

## MATERIALS AND METHODS

One experiment repeated over two seasons was conducted at the Estación Experimental J. Hirschhorn, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, in the east-central region of Argentina. We collected data over 2 yr, beginning with the 2002/2003 crop season and continuing with the 2003/2004 crop season, on a field grown with wheat for the previous three seasons (1999–2001). In 1999, field plots were established using either conventional tillage or zero tillage in a wheat crop. The experiment was moved in the second season to different plots grown next to the experiment in the 2002 season, an area which had also been planted to wheat in zero tillage or conventional tillage during the 2002 season. Conventional tillage consisted of working the field twice with a plow and twice with a harrow to a depth of 15 cm. Before sowing, zero tillage plots received one application of glyphosate (N-(phosphonomethyl), glycine) at a rate of 2 L ha<sup>-1</sup> (formulated product) to control weeds and volunteer wheat.

The soil was a typical Argiudoll (loamy). Analysis of soil samples (top 0.20 m) indicated the following values by weight: organic matter = 4.21%; N = 0.21%; NO<sub>3</sub> = 76.6 mg kg<sup>-1</sup>; P (Bray Kurtz) = 6.7 mg kg<sup>-1</sup>, and pH = 6.1 for one composite sample analyzed for the conventional tillage plots, and organic matter = 4.38%; N = 0.21%; NO<sub>3</sub> = 42 mg kg<sup>-1</sup>; P (Bray Kurtz) = 6 mg kg<sup>-1</sup>, and pH = 6.1 for the zero tillage plots for the 2002 season. The analysis indicated the following values by weight for 2003 season = organic matter 4.11%; N = 0.20%; NO<sub>3</sub> = 72 mg kg<sup>-1</sup>; P = 6.6 mg kg<sup>-1</sup>, and pH = 6.1 for the conventional tillage plots, and organic matter = 4.40%; N = 0.21%; NO<sub>3</sub> = 39 mg kg<sup>-1</sup>, P = 5.8 mg kg<sup>-1</sup> and pH 6.1 for the zero tillage plots. The trials were sown on 8 Aug. 2002 and 16 July 2003. The experimental design was a split-split-split plot, in randomized complete blocks with three replications

(blocks). The plots in each experiment were sown with a direct drill, a Deutz Agroline DS-4300, with a row spacing of 17.5 cm and a seeding rate of 350 seeds  $m^{-2}$ .

Main plots were the tillage treatments: conventional tillage and zero tillage, where wheat had been managed under the same systems (conventional or zero tillage) since 1999. Main plots were spaced 5 m apart to minimize interplot interference. Subplots were the cultivars Buck Pingo, Buck Bigua, and Buck Brasil (seeds provided by its breeder, Buck S.A.) which differ in resistance to several foliar diseases. Buck Bigua and Buck Pingo are moderately resistant to tan spot and Septoria blotch, while Buck Brasil is moderately susceptible to these two diseases; Buck Pingo is more susceptible than the other cultivars to leaf rust. All cultivars are early heading, averaging 94, 95, and 97 d from emergence to heading for Buck Bigua, Buck Brasil, and Buck Pingo, respectively. The sub-subplots were the N fertilization treatments (0, 80, and 160  $kg\ ha^{-1}$  N) in a split application: (i) no N applied; (ii) 40  $kg\ ha^{-1}$  N at sowing followed by 40  $kg\ ha^{-1}$  N at GS 32 (Zadoks et al., 1974); and (iii) 80  $kg\ ha^{-1}$  N at sowing followed by 80  $kg\ ha^{-1}$  N at GS 32. Fertilization rates of 160  $kg\ ha^{-1}$  N are the greatest N rates used in farmer fields. The sub-sub-subplots were a foliar fungicide treatment and an untreated control. Plots were treated with Caramba fungicide [(metconazole 9%), Bayer Corp. Leverkusen, Germany] at a rate of 1 L product in 150 L water  $ha^{-1}$  at GS 22 (on 20 Sept. 2002 and 10 Sept. 2003) and again at GS 39 (on 24 Oct. 2002 and 17 Oct. 2003) with a flat fan spray nozzle at 400 kPa. Although GS 39 is the most usual growth stage for fungicide application, GS 22 is also used when early foliar disease is evident. Plots were 3.67 m wide by 5 m long (21 rows per plot). Each season, the entire trial area was fertilized with 100  $kg\ ha^{-1}$  of calcium triple superphosphate (Triavet S.A., Argentina) at the time of sowing. Glyphosate (2 L  $ha^{-1}$ ) in 120 L water was applied in the zero tillage plots 15 d before sowing. In addition, Misil herbicide [(metsulfuron metil dry flowable 60% + Dicamba soluble liquid 57.1%, Dupont, Rosario, Argentina)] at 100  $cm^3$  metsulfuron metil + 6.7 g Dicamba (dimethylamine 2- metoxi-3,6 diclorobenzoic acid) in 120 L water  $ha^{-1}$  was applied to the entire experiment at the three leaf stage in all plots in each season.

Environmental variables (total monthly precipitation; relative humidity; minimum, maximum, and mean daily temperatures) were recorded at a station situated 100 m from the experiment.

### Foliar Disease Severity and Biomass Determination

The percentage of chlorotic and necrotic area of wheat leaves in each plot was used as an indicator of the amount of damage caused by foliar diseases. Percent chlorosis and necrosis were visually estimated on the upper four leaves of 12 plants per plot at GS 23 (on 30 Sept. 2002 and 20 Sept. 2003) and on the upper two leaves of 12 plants per plot at GS 49, 70, and 83 (on 31 Oct. and 15 and 28 Nov. in 2002, and 24 Oct. and 7 and 22 Nov. in 2003). Each evaluation was performed over 3 d because of slight differences when each cultivar reached the right growth stage. Visual estimates of foliar severity were calibrated by comparing a subsample with the data obtained using Optimas image system analysis (Media Cybernetics, Silver Spring,

MD 20910). The area under disease progress curve (AUDPC) was calculated with disease ratings from the last three growth stages, according to Shaner and Finney (1977). Aboveground crop biomass was measured for two of the three cultivars (Buck Pingo and Buck Bigua). Aboveground biomass was measured at GS 23 and GS 83 by removing all aboveground plant material from three 0.50-m long sections taken from the center of each plot. The crop material was dried at 70°C for 48 h and then weighed.

### Yield and Yield Components

Four meters from the three center rows in each plot (2.10  $m^2$ ) were hand-harvested, threshed, and the kernel yield ( $kg\ ha^{-1}$ ) calculated. Yield components—SPM2, KPS, and TKW—were calculated from each plot. In addition, three 1-m sections were harvested from the three center rows (0.525  $m^2$ ) and the spikes counted to determine SPM2. From that sample, KPS was determined for 30 spikes, then heads were threshed, and kernels counted. The kernels counted in the 30 spikes were weighed to calculate TKW.

### Frequency of Foliar Pathogens

To evaluate the predominant fungal pathogens causing foliar disease, approximately 600 green leaves in total were collected roughly equally across all plots with symptoms indicative of foliar disease. Leaves were scored for the presence of leaf and stripe rust using a hand lens. In addition, leaves were gently washed under tap water and cut into five pieces each approximately 1  $cm^2$ . These pieces were surface sterilized by dipping successively into 70% ethanol for 1 min, 5% sodium hypochlorite (Clorox chlorine solution at 55  $g\ L^{-1}$  Cl) for 3 min, and rinsed twice in sterile, distilled water. Leaf pieces were placed in Petri dishes containing 3.9% potato dextrose agar supplemented with 500  $mg\ L^{-1}$  chloramphenicol. The dishes were incubated in the darkness at 26°C for 9 d and observed every 2 to 3 d. Fungal species (or genus for *Alternaria* spp.) were identified based on cultural characteristics and the morphology of fruiting bodies and spores (Zillinsky, 1983). The frequency of leaf section infection was calculated per plot as the number of leaf sections from which a given fungus was isolated divided by the total number of leaf sections incubated.

### Statistical Analysis

Data were analyzed by ANOVA for a split-split-split plot design within a randomized complete block design with a combined analysis across both seasons and within each season separately because of the significance of year effects and/or interactions of seasons with other factors in the ANOVA (SPSS for Windows, sixth edition, IBM, New York). Homogeneity of variance was tested by comparing the error mean squares for all factors for all dependent variables (Snedecor and Cochran, 1989). Variances were homogeneous and factors were considered as fixed, except replications that were considered as random. Means were compared by Fisher's Protected Least Significant Difference (LSD) ( $P = 0.05$ ). Linear multiple regression analyses were calculated between foliar disease severity values as the dependent variables and weather conditions as the independent variables. The dependent variable was AUDPC values for each season, and independent variables were the

**Table 1. Environmental conditions during the wheat growing seasons of 2002–2003 and 2003–2004 at Los Hornos, La Plata, Argentina**

Month	Monthly mean temp.		Monthly max. temp.		Monthly min. temp.		Monthly total radiation		Monthly total precip.		Monthly relative humidity	
	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003
	°C											
	Watt m <sup>-2</sup>											
	mm											
	%											
August	12.1	9.7	17.3	14.8	7.4	5.0	2515	2489	90.6	27.2	78.3	77.5
September	12.5	12.2	17.5	17.6	7.8	6.8	3137	3346	90.0	81.4	80.4	78.7
October	17.4	16.4	22.5	22.4	12.2	10.4	4080	4875	67.6	61.6	80.5	77.5
November	19.2	18.0	24.5	23.4	13.6	12.0	4962	4981	112.0	160.0	74.0	73.3
December	20.7	19.8	25.8	25.3	15.1	13.7	5306	5518	60.4	55.0	78.4	76.6
Average	16.4	15.2	21.5	20.7	11.2	9.6	4000	4242	420.0	385.0	78.3	76.7

**Table 2. The ANOVA of disease intensity and wheat biomass over two seasons at Los Hornos, La Plata, Argentina.**

Source of variation	df	Disease severity				Biomass					
		GS 23		AUDPC		GS 23		GS 83			
		2002	2003	2002	2003	2002	2003	2002	2003		
		<i>P</i> > <i>F</i>									
Tillage system (T)	1	0.041	0.037	<0.001	0.045	0.050	0.026	0.029	0.002		
Error a	2										
Cultivar (C)	2	0.081	0.253	<0.001	0.031	0.004	0.010	0.032	0.006		
T × C	2	0.251	0.708	0.961	0.831	0.779	0.298	0.419	0.056		
Error b	8										
N fertilization (N)	2	0.017	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
T × N	2	0.229	0.051	0.601	0.291	0.908	0.551	0.439	0.217		
C × N	4	0.371	0.537	0.587	0.786	0.493	0.147	0.053	0.070		
T × C × N	4	0.063	0.393	0.977	0.599	0.423	0.566	0.662	0.340		
Error c	24										
Fungicide (F)	1	<0.001	<0.001	<0.001	<0.001	0.033	0.072	<0.001	<0.001		
T × F	1	0.002	0.130	0.125	0.913	0.544	0.686	0.649	0.396		
C × F	2	0.360	0.319	0.557	0.301	0.370	0.241	0.665	0.203		
N × F	2	0.334	0.210	0.285	0.440	0.056	0.238	0.056	0.418		
T × C × F	2	0.523	0.933	0.056	0.084	0.906	0.882	0.498	0.748		
T × N × F	2	0.088	0.414	0.307	0.364	0.975	0.103	0.870	0.054		
C × N × F	4	0.367	0.056	0.165	0.076	0.373	0.700	0.605	0.698		
T × C × N × F	4	0.584	0.436	0.604	0.371	0.755	0.552	0.272	0.691		
Error d	36										

**Table 3. Means of disease intensity and wheat biomass over two seasons at Los Hornos, La Plata, Argentina.**

Treatments	Disease severity (%)				Biomass (g m <sup>-2</sup> ) GS			
	GS 23		AUDPC		23		GS 83	
	2002	2003	2002	2003	2002	2003	2002	2003
Season	10.5	14.7	1135	1569	90	77	999	1058
<b>Tillage systems</b>								
Conventional tillage	7.5a†	9.0a	1009a	1455a	93a	85b	1045b	1180b
Zero tillage	13.7b	20.3b	1261b	1684b	86a	67a	954a	936a
<b>Cultivars</b>								
Buck Pingo	9.7a	15.4a	1333b	1669b	103b	84b	1045b	1249b
Buck Bigua	9.1a	13.0a	794a	1463a	77a	70a	944a	1091a
Buck Brasil	13.0a	15.6a	1279b	1575ab	–	–	–	–
<b>N fertilization rate</b>								
0 kg ha <sup>-1</sup> N	12.2b	18.4c	1254b	1802c	65a	55a	790a	793a
80 kg ha <sup>-1</sup> N‡	10.5a	14.4b	1112a	1527b	95b	78b	1078b	1123b
160 kg ha <sup>-1</sup> N‡	9.0a	11.2a	1038a	1378a	109c	97c	1230c	1257c
<b>Fungicide treatment</b>								
No fungicide applied	12.4b	18.0b	1398b	1791b	85a	73a	925a	967a
Two fungicide applications§	8.4a	11.3a	873a	1348a	94b	80b	1064b	1148b

† Means followed by the same letter within the same column are not statistically different (LSD *P* < 0.05).

‡ At the time of disease assessment at GS 23 only half the amount of N indicated had been applied.

§ At the time of disease assessment at GS 23 only one fungicide application had been made, and there was 10 d between fungicide application at GS 22 and disease assessment at GS 23.



weather variables measured 30 d before the disease ratings for each season.

## RESULTS

### Environmental Variables

Average temperature from seedling emergence through harvest was 8% higher for the 2002 season than for 2003 season and close to the monthly average temperature of 16.5°C for the region (1979–2009) from August to December (Table 1). Total radiation was 6% lower and total precipitation 8% higher for 2002 compared to 2003. In addition average relative humidity was 3% higher for 2002 compared to 2003. August was especially cooler in 2003 (2.4°C less). Radiation was 20% greater in October 2003 compared to October 2002. Humidity was very high for both seasons in all months (Table 1).

### Disease Severity and Biomass

The ANOVA for each season indicated that tillage system, N fertilization rate, and fungicide treatment significantly influenced foliar disease severity at GS 23 and AUDPC in both seasons (Table 2). In addition cultivars significantly affected the AUDPC in both seasons. The tillage × fungicide interaction term for disease severity at GS 23 in 2002 was also significant, but not in 2003, nor for the AUDPC ratings in either season. No other interaction terms were significant for disease rating at GS 23 or for AUDPC ratings. Foliar disease severity at GS 23 was 39% greater in 2003 than in 2002 (Table 3). At GS 23, conventional tillage reduced foliar disease severity 46% in 2002 and 56% in 2003 compared to zero tillage. Application of N reduced foliar disease severity at GS 23 (Table 3). Disease severity at GS 23 was similar on each cultivar. The AUDPC values were 38% greater in 2003 than in 2002 (Table 3). The AUDPC values were 20 and 14% lower in conventional tillage than zero tillage plots for 2002 and 2003, respectively. In the adult stage, Buck Bigua was more resistant to disease development than the other cultivars (as indicated by its AUDPC values) in 2002, and more resistant than Buck Pingo in 2003. Nitrogen fertilizer reduced AUDPC in both years (Table 3, Fig. 1 and 2). Tillage system, cultivar, and N fertilization rate significantly affected crop biomass at both GS 23 and GS 83 in both seasons (Table 2). Fungicide application increased biomass at GS 23 in

2002 and GS 83 in 2002 and 2003, probably as a result of the significant decrease in foliar disease severity

There was no significant interaction for any of the four main factors for crop biomass at either GS 23 or GS 83 in either season (Table 2). Biomass was 16% greater at GS 23 in 2002 and 6% greater at GS 83 in 2003, probably due to the greater total precipitation at the beginning (August) of 2002 (Table 3). Buck Pingo had the greatest biomass in both seasons. Nitrogen fertilizer and fungicide increased crop biomass at both GS 23 and GS 83 (Table 3).

The significant interaction of tillage and fungicide treatment on disease severity at GS 23 in 2002 (Table 2) was because fungicide had less effect on foliar disease severity in conventional tillage plots than in zero tillage (Table 4). In conventional tillage without fungicide applications, foliar disease severity was less compared to zero tillage. Zero tillage increased disease severity ratings for all three cultivars similarly. No other interactions terms were significant (Table 2).

### Yield and Yield Components

Yield was affected significantly by the three way seasons × tillage × N interaction. Due to the significant season interactions, a separate ANOVA was conducted for each season (Table 5). Nitrogen rate and fungicides significantly affected yield in both years, but tillage was significant only in 2003. Yields were greater in conventional tillage plots than in zero tillage plots in 2003. Nitrogen rates and fungicides increased yield in both years. Cultivars did not differ significantly in yield (Table 6). Rate of N had a large effect on yield in both conventional and zero tillage, as did fungicide application (Table 7).

Although yield components (SPM2, KPS, and TKW) significantly differed for each cultivar, the components compensated for each other. Buck Pingo had more SPM2 and heavier kernels but fewer KPS, whereas Buck Brasil and Buck Bigua had fewer SPM2 than Buck Pingo but more KPS in both seasons. Buck Pingo had the heaviest kernels in both seasons. Nitrogen increased SPM2 and KPS significantly (Table 6). Nitrogen increased TKW significantly in 2002 but not in 2003. The increased yield that resulted from foliar fungicide application resulted from increases in SPM2 and TKW in both years, and also from KPS in 2003. Several interactions were significant for yield component data (Table 5). The response of SPM2 to N

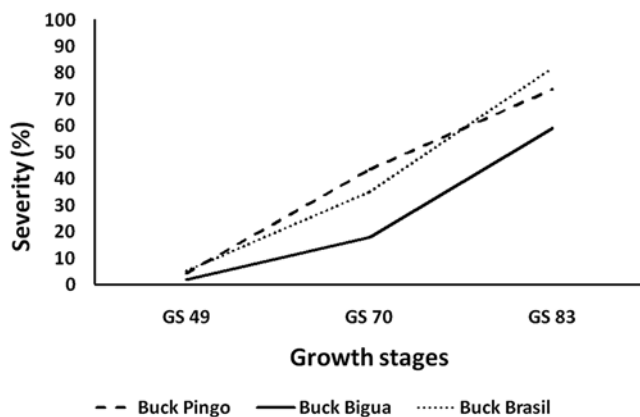


Fig. 1. Area under disease progress curve (AUDPC) values for the 2002–2003 season for disease on three wheat cultivars that received three N fertilization rates without fungicides (La Plata, Argentina).

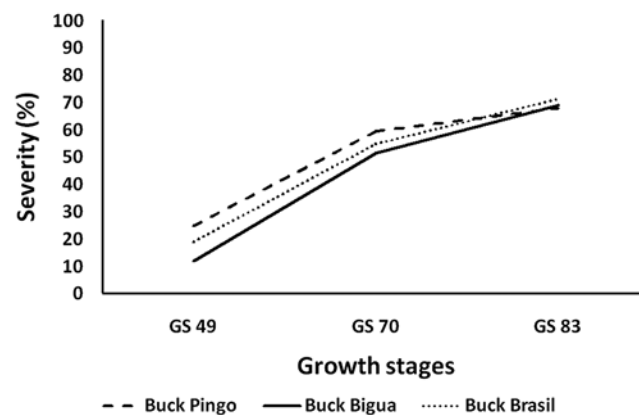


Fig. 2. Area under disease progress curve (AUDPC) values for the 2003–2004 season for disease on three wheat cultivars that received three N fertilization rates without fungicides (La Plata, Argentina).

**Table 4. Means for the interactions of cultural practices on foliar disease intensity and wheat biomass over two season at Los Hornos, La Plata, Argentina.†**

Fungicide treatment	2002								2003							
	Conventional tillage				Zero tillage				Conventional tillage				Zero tillage			
	0N	80N	160N	Avg.	0N	80N	160N	Avg.	0N	80N	160N	Avg.	0N	80N	160N	Avg.
Disease severity GS 23, %‡																
Without fungicide	8.2	7.3	8.4	8.0	18.4	18.5	13.4	16.8	14.6	13.0	7.3	11.6	30.9	22.9	19.5	24.4
With fungicide	7.8	7.3	5.8	7.0	14.3	9.1	8.3	10.6	6.9	6.2	6.1	6.4	21.2	15.6	11.9	16.2
Averages	8.0	7.3	7.1	7.5	16.3	13.8	10.9	13.7	10.8	9.6	6.7	9.0	26.1	19.3	20.3	21.9
AUDPC‡																
Without fungicide	1364	1197	1189	1250	1610	1569	1456	1545	1920	1590	1525	1678	2098	1885	1728	1904
With fungicide	943	751	610	768	1102	934	897	978	1450	1171	1074	1232	1742	1465	1184	1464
Averages	1153	974	899	1009	1356	1251	1176	1261	1685	1380	1299	1455	1920	1675	1456	1684
Biomass (g) GS 23																
Without fungicide	66.7	84.4	109	86.7	61.9	82.2	106	83.4	70.2	85.7	94.1	83.3	42.4	60.3	86.9	63.2
With fungicide	72.1	110	115	99.0	59.4	104	105	89.5	65.1	83.7	117	88.6	43.8	80.7	90.1	71.5
Averages	69.4	97.5	112	92.9	60.7	93.1	105	86.4	67.6	84.7	106	86.0	43.1	70.5	88.5	67.4
Biomass (g) GS 83																
Without fungicide	688	1090	1113	964	629	951	1082	887	911	1100	1197	1069	561	882	1153	865
With fungicide	762	1202	1416	1127	683	1069	1309	1020	898	1464	1509	1290	802	1047	1169	1006
Averages	725	1146	1265	1045	656	1010	1196	953	904	1282	1353	1179	682	964	1161	936

† Only significant LSD ( $P = 0.05$ ) values are given. LSD interaction tillage system (T) × fungicide (F) severity GS 23, 2002 = 5.82.

‡ AUDPC; area under disease progress curve, GS, growth stage.

between conventional and zero tillage differed between the two growing seasons (Table 7). No other interaction terms were significant (Table 5).

### Foliar Diseases Frequency

The predominant pathogen causing foliar disease in this study was *Pyrenophora tritici-repentis*, with incidence (frequency isolation from leaf pieces) of 36% in 2002 and 57% in 2003, followed by pathogenic *Alternaria* spp. with incidence of 29 and 16%, respectively, and *Bipolaris* spp. with incidence of 6 and 8% for each season, respectively. Stripe rust was not observed and the incidence of leaf rust was low (<2%).

Linear regression models between AUDPC and weather variables showed that the best model to predict AUDPC values was:  $AUDPC = 0.14 P (SE = 0.299) + 6.90 T (SE = 0.005) - 87.44$ ,  $R_2 = 0.86$ , which was significant at  $P < 0.001$  in which  $P =$  sum of precipitation (mm) for the entire period of 1 mo before GS 23; GS 49; GS 70 and GS 83 and  $T =$  mean temperature ( $^{\circ}C$ ) for the same period.

### DISCUSSION Foliar Disease Severity

In this study, the use of zero tillage under monoculture increased foliar disease severity on wheat (caused mainly by the

**Table 5. The ANOVA of yield and yield components of wheat over two seasons at Los Hornos, La Plata, Argentina.**

Source of variation	df	SPM2†		KPS†		TKW†		Yield	
		2002	2003	2002	2003	2002	2003	2002	2003
$P > F$									
Tillage system (T)	1	0.271	0.289	0.679	0.321	0.168	0.255	0.358	0.004
Error a	2								
Cultivar (C)	2	0.006	<0.001	0.003	<0.001	<0.001	<0.001	0.562	0.130
T × C	2	0.228	0.082	0.301	0.413	0.204	0.100	0.303	0.459
Error b	8								
N	2	<0.001	<0.001	<0.001	<0.001	0.095	0.488	<0.001	<0.001
T × N	2	0.050	0.009	0.664	0.153	0.010	0.488	0.590	0.008
C × N	4	0.464	0.152	0.132	<0.001	0.011	0.729	0.068	0.577
T × C × N	4	0.516	0.245	0.624	0.064	0.593	0.673	0.427	0.532
Error c	24								
Fungicide (F)	1	0.052	0.043	0.994	0.016	<0.001	0.001	<0.001	<0.001
T × F	1	0.089	0.944	0.019	0.569	0.121	0.056	0.742	0.506
C × F	2	0.762	0.156	0.599	0.103	0.571	0.582	0.772	0.756
N × F	2	0.464	0.334	0.116	0.149	0.673	0.765	0.364	0.199
T × C × F	2	0.999	0.337	0.844	0.100	0.105	0.538	0.931	0.595
T × N × F	2	0.807	0.571	0.544	0.255	0.985	0.293	0.150	0.654
C × N × F	4	0.422	0.004	0.821	0.970	0.232	0.405	0.452	0.427
T × C × N × F	4	0.954	0.105	0.198	0.999	0.480	0.962	0.324	0.217
Error d	36								

† SPM2, spike per square meter; KPS, kernels per spike; TKW, thousand kernel weight.

**Table 6. Means of yield and yield components of wheat over two seasons at Los Hornos, La Plata, Argentina.**

Treatments	Yield, kg ha <sup>-1</sup>		SPM2† (no.) SPM2 (nN)		KPS (no.) KK		TKW (g)	
	2002	2003	2002	2003	2002	2003	2002	2003
	Seasons	5348	4149	421	382	33.3	29.2	37.6
<u>Tillage systems</u>								
Conventional tillage	5536a‡	4396b	431a	394a	33.5a	29.6a	38.3a	37.9a
Zero tillage	5160a	3903a	411a	370a	33.2a	28.8a	36.9a	35.8a
<u>Cultivars</u>								
Buck Pingo	5395a	4754a	470b	428b	29.8a	26.4a	39.3c	38.8b
Buck Bigua	5456a	3954a	414a	369a	34.0b	30.2b	37.5b	35.2a
Buck Brasil	5193a	3969a	394a	349a	36.1b	31b	35.6a	35.8a
<u>N fertilization rate</u>								
0 kg ha <sup>-1</sup> N	3872a	2615a	343a	302a	30.2a	24.0a	37.0a	36.3a
80 kg ha <sup>-1</sup> N	5727b	4392b	440b	392b	34.3b	30.7b	37.7b	36.4a
160 kg ha <sup>-1</sup> N	6444c	5441c	478c	452c	35.4c	32.7c	37.9b	37.3a
<u>Fungicide treatment</u>								
No fungicides applied	5471a	3899a	411a	375a	33.3a	28.3a	36.4a	36.0a
Two fungicides applications	5882b	4400b	430b	390b	33.3a	30.1b	38.3b	37.6b

† SPM2, spike per square meter; KPS, kernels per spike.

‡ Means followed by the same letter within the same column are not statistically different (LSD P = 0.05).

necrotrophic pathogen *Pyrenophora tritici-repentis*) possibly due to increased residue levels. This agrees with other studies (Sutton and Vyn, 1990; Bockus and Claassen, 1992; Fernandez et al., 1998, 1999; Carignano et al., 2008). However, Bailey et al. (1992, 1995, 2000, 2001) reported that reduced tillage did not substantially increase the risk of crop losses due to foliar diseases. Differences in results among these studies probably reflect the fact that Bailey et al.'s experiments were conducted under crop rotations rather than monoculture, as was the case for the other cited studies. Bockus and Claassen (1992) reported that rotation was as effective as plowing for control of tan spot. *Pyrenophora tritici-repentis* forms ascospores and conidia, both of which can serve as inoculum. Ascospores are dispersed relatively short distances while conidia can be widely dispersed (Schilder and Bergstrom, 1992). Crop rotations are, apparently, essential for reducing the number of ascospores

in wheat residues in regions where ascospores are important inoculum early in the season.

Environmental conditions also play an important role in the effect of tillage practices on the severity of foliar diseases in wheat. This study was conducted in a humid region of Argentina, where mean total precipitation (average from 1979–2009) for the period of August to December (the wheat growing season) is typically 409 mm. During this study, rainfall was greater than this average in 2002 (420 mm) and slightly less in 2003 (384 mm). In addition the increase in precipitation in 2002 was associated with a reduction in total radiation during that year. Only in August 2003 was rainfall much less than the 30-yr average for that month. A combination of zero tillage and greater precipitation greatly increases foliar disease severity (Krupinsky et al., 2002, 2004, 2007a; Krupinsky and Tanaka, 2001). On the contrary, in a study done in a semiarid region,

**Table 7. Means for the interactions of cultural practices on yield and yield components of wheat over two seasons, at Los Hornos, La Plata, Argentina.†**

Fungicide treatment	2002								2003							
	Conventional tillage				Zero tillage				Conventional tillage				Zero tillage			
	0N	80N	160N	Avg.	0N	80N	160N	Avg.	0N	80N	160N	Avg.	0N	80N	160N	Avg.
Yield, kg ha <sup>-1</sup>																
Without fungicide	3918	5684	6347	5316	3469	5223	6005	4899	2617	4639	5045	4100	2180	3314	5602	3699
With fungicide	4040	6037	7192	5756	4063	5964	6235	5421	3278	5397	5399	4691	2384	4220	5720	4108
Averages	3979	5861	6769	5536	3766	5593	6120	5160	2948	5018	5222	4396	2282	3767	5661	3903
SPM2 (no.)																
Without fungicide	356	426	506	429	323	419	439	394	307	411	444	387	293	345	450	363
With fungicide	342	442	512	432	353	472	460	428	312	450	444	402	298	366	470	378
Averages	349	434	509	431	338	445	449	411	309	430	444	394	295	355	460	370
KPS (no.)																
Without fungicide	29.1	35.5	33.9	32.8	30.7	34.7	36.0	33.8	23.5	31.5	31.6	28.9	21.2	28.1	33.9	27.7
With fungicide	30.9	34.6	37.0	34.2	30.2	32.5	34.8	32.5	26.3	31.7	32.7	30.2	25.1	31.8	32.8	29.9
Averages	30.0	35.0	35.5	33.5	30.5	33.6	35.4	33.3	24.9	31.6	32.1	29.6	23.2	29.9	33.3	28.8
TKW, g																
Without fungicide	37.6	38.2	37.4	37.7	34.6	35.9	37.1	35.9	35.3	36.4	36.6	36.1	35.4	34.3	37.0	35.6
With fungicide	38.9	39.1	38.2	38.7	37.1	37.7	38.8	37.9	39.9	39.0	38.7	39.1	34.9	36.1	37.0	36.0
Averages	38.2	38.6	37.8	38.2	35.8	36.8	38.0	36.9	37.6	37.7	37.7	37.7	35.1	35.2	37.0	35.8

† Only significant LSD values are given (P = 0.05). Spike per square meter (SPM2): LSD tillage system (T) × N, 2002 = 74; 2003 = 96; kernels per spike (KPS): LSD T × fungicide (F), 2002 = 3.8; cultivar (C) × N, 2003 = 8.4; thousand kernel weight (TKW): LSD T × N, 2002 = 2.7; C × N, 2002 = 2.8; Yield: LSD T × N, 2003 = 1742.

no significant effect of tillage systems on leaf spot severity was observed (Fernandez et al., 1998), which was attributed to a lower density of pseudothecia in zero tillage plots than in conventional tillage plots, and was probably also affected by the inhibitory effect of glyphosate on the formation of these structures (Sharma et al., 1989). Stover et al. (1996) reported that although foliar diseases were more severe early in the season compared to later when wheat was planted after chisel plowing of a previous wheat crop, those effects were not consistent when considering the AUDPC values in the adult stage. In our study, the increase in foliar disease severity in zero tillage plots compared to conventional tillage plots was greater early in the growing season (GS 23) (increase of 105% on average for both seasons), although AUDPC values also increased significantly (19% on average for both seasons) in zero tillage compared to conventional tillage plots, probably as a result of greater inoculum levels early in the growing season. In this study, the severity of foliar diseases was greater in 2003 than in 2002. Although weather conditions were more conducive to disease development mainly at the beginning of 2002, the greater severity in 2003 may have been because of more inoculum due to an additional year of wheat monoculture for 2003 compared to 2002, more favorable weather conditions for survival of inoculum between crops, or the increased precipitation at the end of the growing season. Total precipitation and mean temperature 1 mo before each disease evaluation explained 87% of the variation in disease severity through the different growth stages, treatments, and growing seasons.

Nitrogen fertilization at 80 or 160 kg ha<sup>-1</sup> N decreased the severity of foliar diseases on wheat in this study. Krupinsky and Tanaka (2001) and Krupinsky et al. (2004) reported similar results in experiments where tan spot was the predominant disease. When *Septoria* leaf blotch is the main disease, however, results are inconsistent; previous studies (Cox et al., 1989; Howard et al., 1994; Leitch and Jenkins, 1995; Simón et al., 2002, 2003) determined that N fertilization increased the severity of *Septoria* leaf blotch under conducive weather conditions (mean temperature from 17 to 24°C, high relative humidity and precipitation). Hayden et al. (1994) did not find differences between N fertilization treatments and Tompkins et al. (1993) found an increase in *Septoria* leaf blotch severity with low N fertilization in one of his experiments

Differences in foliar disease resistance among three cultivars were reflected by AUDPC values in this study. Buck Bigua was the most resistant of the three cultivars. Carignano et al. (2008) reported lower differences in disease severity between zero tillage and conventional tillage treatments, and lower yield response to fungicide application for a resistant cultivar than for a susceptible cultivar than what we found in our study. However, differences in resistance among the three cultivars we evaluated were not as great as those reported by Carignano et al. (2008). The cultivars in our study ranged from susceptible to only moderately susceptible to tan spot, whereas the study by Carignano et al. (2008) included resistant cultivars. Although fungicide application had a more significant effect than N fertilizer rates at controlling the foliar disease complex considering the average of both seasons in this study, N fertilizer also had a significant effect. The AUDPC values were reduced in plots that received 160 kg ha<sup>-1</sup> N compared to 0 kg ha<sup>-1</sup> N as

much as by fungicide applications in 2003 compared to plots with no fungicide applications.

### **Effects of Foliar Diseases on the Crop Biomass, Yield, and Yield Components**

Foliar diseases significantly reduced the crop biomass during both growing seasons and fungicide application increased crop biomass. These results corroborate other reports (Ruske et al., 2003; Serrago et al., 2009) that observed that fungicide significantly improved crop biomass.

Wheat yields were lower in 2003 than in 2002, probably due mainly to less precipitation at the beginning of the 2003 crop season (July and August) and probably also to one more year of continuous wheat compared to the 2002 season. For these reasons there was also significantly reduced crop biomass at GS 23, KPS, and SPM2 that led to reduced yield in 2003 compared to 2002. Yield did increase significantly in association with crop biomass in plots treated with fungicides not fertilized or receiving 80 kg ha<sup>-1</sup> N, but not at 160 kg ha<sup>-1</sup> N. At 160 kg ha<sup>-1</sup> N, the crop biomass was significantly greater at lower rates of fertilizer, but the increase in biomass associated with fungicide application did not increase yield significantly. This indicated that with higher rates of N fertilizer application, the resulting increase in biomass could compensate for the effects of foliar diseases on yield. Carretero et al. (2006) compared fungicide treatments at different N rates in wheat and reported that fungicide application without N fertilization increased the intercepted photosynthetic active radiation (IPAR) by 45%, whereas fungicide applications to plots that also received N fertilization increased IPAR only 25%. Differences in IPAR for plots with and without fungicide treatments were lower in the N fertilized plots probably due to the increase in the biomass. However, Serrago et al. (2009) reported that differences in accumulated absorbed radiation, biomass at harvest, and yield between healthy and diseased plots of wheat were greater in plots that received N applications than in plots not treated with N fertilizer. This could have been caused by the presence of leaf rust which increased under N fertilization.

In this study, two fungicide applications to the foliage at GS 22 and GS 39 increased yield 411 and 501 kg ha<sup>-1</sup> in 2002 and 2003, respectively. Serrago et al. (2009) indicated that a complex of diseases formed by tan spot, *Septoria* leaf blotch and rust reduced grain yield by 1020 kg ha<sup>-1</sup> on average. In the later study disease severity started generally after anthesis and nongreen area fluctuated between 20 and 100% at 40 d after anthesis. In our study *Septoria* leaf blotch started before anthesis and was about 70% at GS 83. This would indicate that the higher yield reduction in the former study could be at least in part attributed to the presence of other diseases as leaf rust.

In our study, at 160 kg ha<sup>-1</sup> N rates, yield reduction caused by foliar diseases was minimized. This indicates a benefit of using adequate N fertilizer, especially with zero tillage practices when tan spot increases. Tebuconazole applied at heading increased yield at high N rate (194 kg ha<sup>-1</sup> N) by an average of 10% (773 kg ha<sup>-1</sup>), but also improved yields significantly in 1 yr in plots with a low (67 kg ha<sup>-1</sup> N) rate (Varga et al., 2005). Differences in the wheat yield response to fungicide application with or without N fertilizer could be associated with the presence of different foliar diseases. When leaf rust was prevalent,



N treatments increased the rust severity (Howard et al., 1994); when Septoria leaf blotch was prevalent, N treatments increased, had no influence, or slightly reduced disease severity (Leitch and Jenkins, 1995; Simón et al., 2002, 2003). When tan spot was prevalent, as in this study, application of N fertilizers generally decreased disease severity (Bockus and Davis, 1993). Due to this and the effect of N on biomass, differences in IPAR, biomass, or yield could be reduced in plots receiving N applications compared to plots not treated with N fertilizers between healthy and diseased crops.

Nitrogen and fungicide applications increased the number of wheat SPM2. Shabeer and Bockus (1988) and Carignano et al. (2008) reported that SPM2 was unaffected by tan spot. In our study, under monoculture, the tan spot inoculum pressure was high from the beginning of the crop season and caused early infection at GS 23. In contrast to these other studies, we applied fungicides earlier (from GS 22 rather than GS 39 in these other studies). In our study SPM2 had not yet been determined at the onset of disease symptoms. As SPM2 increased, KPE were significantly increased only in one of the two seasons with fungicide application. Compensation between yield components has also been reported by other researchers (Carr et al., 2003; Carignano et al., 2008). Applying fungicide to foliage modifies the response of the yield components compared to plots with no fungicide applications. Rees and Platz (1982) and Shabeer and Bockus (1988) reported that fungicide treatments increased KPS compared to plots with no fungicide applications, but Kelley (2001) and Carignano et al. (2008) suggested that KPS was unaffected by tan spot because this yield component was determined before the fungicide was applied. Thousand kernel weight was also increased significantly by fungicide treatments in our study; similar to the results of Kelley (1993, 2001) and Carignano et al. (2008) with a susceptible wheat cultivar, but not a resistant cultivar.

### Foliar Disease Frequency

The type of disease influences the effect of tillage, N, and fungicide treatment on severity and yield loss (Bockus and Davis, 1993; Cox et al., 1989; Howard et al., 1994; Leitch and Jenkins, 1995; Simón et al., 2002, 2003). Because the effect of these practices is influenced by the type of disease, it is important to know what are the prevalent diseases in each area. In our study tan spot was the most prevalent disease, followed by *Alternaria* leaf blight and spot blotch caused by *Bipolaris* spp. Krupinsky et al. (2007a), reported that *P. tritici-repentis* and *S. nodorum* were the major components of the foliar disease complex, followed by *B. sorokiniana* in a study conducted at Mandan, ND. Carignano et al. (2008) reported that *Blumeria graminis tritici*, causal agent of powdery mildew, and *P. striiformis*, causal agent of stripe rust, occurred in addition to tan spot in a study in Marshall County, Kansas. Fernandez et al. (1999) and Jorgensen and Olsen (2007) reported that *P. tritici-repentis* was the most prevalent leaf pathogen of wheat in their studies in Saskatchewan, Canada, and Flakkebjerg, Denmark, respectively. Tan spot is also an important disease in zero tillage systems in Queensland, Australia, and can cause yield losses as great as 75% (Rees and Platz, 1982). Varga et al. (2005) reported greater frequencies of *P. triticina* and *P. striiformis*, followed by *S. tritici* and *B. graminis-tritici* at Zagreb, Croatia.

Although *P. triticina* is an important pathogen in the Argentinean Pampas, this pathogen was at low incidence in our study, due in part to the high frequency of *P. tritici-repentis*. The presence of *P. striiformis* is not usual in this region (central part of the wheat growing area of Argentina), but is more important in the southern part of the Argentinean wheat-growing region where temperature is lower. This study also showed the importance of *Alternaria* spp., a necrotrophic complex that is increasing in prevalence in the entire Argentinean wheat-growing region (Perelló and Moreno, 2004). Although the diseases caused by these pathogens have not been studied well yet, we expect, that they would respond similarly as tan spot to N and zero tillage because they are also necrotrophic pathogens. In general when tan spot was the prevalent disease, N fertilization decreased the intensity of the disease; when *P. triticina* was prevalent, N fertilization increased it. As we found in our study, Carignano et al. (2008) found that high N fertilization rates produced larger grain yields. Varga et al. (2005), in a study in which *P. triticina* was the prevalent pathogen, reported that high rates of N increased yield, except when disease was severe and no fungicide was applied, in which case the high rate of N provided no benefit over the low rate.

As zero tillage is a well-established practice in Argentina and other countries, management of wheat crops, including the use of cultivar resistance, appropriate N fertilization rates, and fungicide applications should minimize severity of tan spot in wheat monoculture systems.

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