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Recent Advances on Integrated Foliar Disease Management with Special Emphasis in Argentina Wheat Production

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1. Introduction

Wheat (*Triticum aestivum* L.) is grown in most regions of the globe due to its importance as a food source, and its enormous genetic variability in phenological response to photoperiod and temperature including vernalization [1]. Argentina is one of the countries with the largest wheat-growing area with more than 5 million ha spread all over the country.

Most of the Argentinean wheat is produced in the Pampean region. This region has a temperate humid climate without a dry season and with a warm summer. Precipitation is higher in summer than in winter. The rainfall distribution is close to monsoonal in the north-west of the Pampas and it tends to an isohigrous pattern at the southeast of Buenos Aires, which means that excess or defect of precipitation could appear at any time. The temperature regimen for the region shows that June and July are the coldest months and January is the hottest. Mean monthly temperatures rarely fall below 7°C and the period of free frost ranges between 180 and 260 days. Temperature indices decrease along a north-south direction, but thermal amplitude also increases from east to west; the frequency and intensity of frost increase westward.

It has an annual rainfall of approximately 600-1000 mm and a mean temperature of 15-17 °C depending on the region, with some differences between the east and the west. Soils in the region are mainly mollisols including argiudolls, hapludolls and haplustolls developed on a deep mass of Pampean loess [2]. Wheat crops are sown from the second half of May to the first half of August. Varieties are classified as long or short season. Long season varieties have higher requirements of long photoperiod or days with low temperatures, although their requirements in vernalization are not as high as in winter varieties cultivated in other countries. Short season varieties have in general low requirements in photoperiod or days

with low temperatures, and are similar to some spring varieties. Risk of frost damage at flowering is the main climatic factor determining optimum sowing dates for particular varieties in the various regions. Optimum seeding rates for long-season varieties may vary between 200 to 250 established plants per m², while short-season varieties tend to be sown with seeding rates between 250 to 350 established plants per m² [3].

During grain production, plant species are rotated following different patterns depending on the region but the most common cropping system tends to be the double-cropped full-season-wheat and soybean [*Glycine max* L. (Merr.)]. The doubled-crop system is usually stable and financially convenient, since wheat crop provides a financial return during summer and the soybean during autumn and winter.

The grain production region has experienced severe tillage changes in the past twenty years, mostly due to the increased interest in maintaining soils covered with plant residues. This has led to implement no tillage systems to restore soil structure in large areas cultivated with double-crop sequences such as wheat/soybean; corn (*Zea mays* L.) - wheat/soybean; or wheat monoculture. No tillage is also desirable because of its positive effect on soil organic matter, for maintaining soil humidity and to prevent soil erosion [4].

No tillage can reduce costs by decreasing fuel consumption required to produce a crop. However, in the wheat/soybean system under no tillage, as in wheat following wheat, the inoculum of necrotrophic fungi may survive until the next wheat season. Therefore, the use of fungicides is essential to decrease the severity of necrotrophic diseases.

On the other hand, nitrogen (N) fertilization is necessary to achieve high yield and grain quality. Even in high soil fertility conditions, N uptake is important because is positively correlated to grain protein content [5]. However, N availability may also enhance the development of some foliar diseases caused by fungi. Fungicides are usually applied on foliage to control diseases but they are also used for seed treatments to prevent seed decay (since soil fungicide applications are not a common practice in Argentina).

2. Wheat yield and quality as affected by foliar diseases

Foliar diseases caused by fungi are the major biotic limitation on yield and quality on wheat [6, 7]. Foliar pathogens reduce yield through reductions in the photosynthesis rate, increasing the rate of respiration, and decreasing translocation of photosynthates from infected tissue [8, 9]. Photosynthesis of diseased plants is reduced due to the destruction of the photosynthetic area. Infected plants usually produce fewer tillers and set fewer grains per spike and the grains are smaller, generally shriveled and of poor milling quality. Shriveled grains occur because the diseases reduce the dry matter destined to the grain but also because the fungi induces earlier maturity of the plant, resulting in decreased time available for the grain to fill [10]. Shriveled grains can contribute to impurities, reduced flour extraction rates and lower contents of metabolizable energy [11].

Foliar pathogens include three diverse groups ranging from poorly specialized necrotroph to highly specialized biotroph parasites. The leaf blights are caused by necrotroph and

hemibiotroph parasitic fungi that cause tissue death. The most important leaf-blight in wheat are tan spot [(*Pyrenophora tritici-repentis* (Died.) Drechs., *Drechslera tritici-repentis* (Died.) Shoemaker)] and Septoria leaf blotch, caused by *Septoria tritici* Rob. ex Desm., teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn. Tan spot symptoms include tan lesions surrounded by a yellow halo on leaves (Fig. 1a), and Septoria leaf blotch produce yellowish speckled leaf spots that later enlarge, turn pale brown and finally dark brown, usually surrounded by a narrow yellow zone (Fig. 1b). Both fungi mentioned before can be grown in laboratory conditions. The control of these foliar diseases by genetic resistance strategies has been difficult because the pathogens have a high variability partially caused by the presence of both asexual and sexual reproduction and because the pathogens show a high degree of specialization. Cultivars in Argentina generally are moderately susceptible to susceptible with only a few with moderate resistance. Therefore, integrated disease management including cultivars with acceptable levels of resistance, crop rotation, seed treatments, different cropping and tillage systems, N fertilization management and fungicides has been used by growers. Tan spot and leaf blotch can be managed by cultural practices such as crop rotation with non-hosts, removal or destruction of infested residue, or tillage, which buries infested residue. Seed treatments are usual since tan spot and leaf blotch can be seed-transmitted, therefore treating seed with fungicide before planting can reduce seed-borne inoculum.

Together with some other pathogenic fungi (mainly *Bipolaris sorokiniana* (Sacc.) Shoem., teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex Dastur and *Alternaria* spp.), tan spot and Septoria leaf 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 [12, 13, 14].



Figure 1. (From left to right): **a.** Tan spot symptoms caused by *Drechslera tritici-repentis* on wheat leaves [15]. **b.** Leaf blotch symptoms caused by *Septoria tritici* [16]. **c.** Leaf rust symptoms caused by *Puccinia triticina* [17].

On the other hand, leaf rust (*Puccinia triticina* Eriks) is the main foliar disease in Argentina caused by a biotroph fungus. It is a very-specialized obligated parasite, thus it cannot be cultivated in laboratory conditions. This foliar disease attacks all the aboveground parts of wheat plants, especially leaves, and causes numerous rusty, orange spots that rupture the

epidermis on wheat leaves (Fig. 1c). Leaf rust may reduce the grain number per plant [18] and the grain produced may be of extremely poor quality, as it may be devoid of starch [10]. Chemical control is a common practice complemented with cultivars with different levels of resistance, usually with a short durability.

3. Different types of fungicides: Its control mechanisms

Planting resistant cultivars is one of the least expensive and most effective management strategies to prevent diseases. However, cultivars with an adequate genetic resistance level to necrotroph foliar diseases are scarce, and usually resistance to leaf rust is complete, conditioned by one or a few genes and has low level of durability in Argentina. Therefore, chemical protection together with cultural practices is a common method of control. In addition, fungicides are also important because Argentinean wheat region combine high yield potential cultivars with high infection pressure, both deriving from adequate temperature and moisture levels, large application of N fertilizers and rotations dominated by cereals, which promote progression of some foliar diseases.

However, the response varies depending not only on the fungicide but also on the N fertilization level, tillage system, foliar disease type and characteristics of the genotypes. The relationship between yield loss and disease severity can differ widely between crop genotypes [9] and some of them exhibit a smaller yield loss under a given severity of infection than others. On the other hand, mechanisms of fungicides to control foliar diseases on wheat may vary according to the active ingredient they have.

Recently, varieties with French germplasm have been introduced or crossed with local germplasm to produce new cultivars in Argentina. These cultivars are characterized by high yield potential but lower resistance to foliar pathogens as tan spot, leaf blotch and leaf rust than the traditional ones. However the increasing adoption by growers of French germplasm varieties susceptible to foliar diseases is leading to a higher use of fungicides.

Triazoles and Strobilurins are the most common systemic fungicides used to control foliar diseases on wheat in Argentina. Statistics shown by Campos [2] indicate that 50% of the products used in Argentina are triazoles and the remaining 50% consists in mixtures of formulations containing triazoles and strobilurins (Fig. 2). Systemic fungicides are absorbed through the foliage or roots and are translocated within the plant through the xylem. These types of fungicides generally move upward in the transpiration stream and may accumulate at the leaf margins [19].

Triazoles are characterized by being an active ergosterol inhibitor, which is the major sterol in fungi. Sterols derivate from terpenes, and they are an essential part of the fungal cell membrane. These molecules are rigid and flat and in its association with the cell membrane give them stability, making it less flexible and allowing the permeability control. Ergosterol Biosynthesis Inhibitors (EBIs) have become one of the most important groups of fungicides, however they may not be effective in controlling Oomycetes because they do not possess the ergosterol synthesis via [19].

The EBIs can be divided into: 1,4 α -demethylase inhibitors (DMIs), which includes the azole (triazole, imidazole) and pyrimidines; the Δ 8,7 isomerase and Δ 1,4 reductase inhibitors (morphines and piperazines) and 3-ceto reductase sterol inhibitors (hydroxyanilide).

The triazoles have been useful to control many foliar diseases. They inhibit the fungus dependent enzyme cytochrome P-450 called 1,4 α -demethylase involved in the ergosterol biosynthesis and consequently affect the permeability of the membrane. However, the mode of action may vary relatively between the different active principles within this group. One of the most common chemicals commercialized in Argentina containing triazoles is Tebuconazole, which is used for seed treatment and foliar and spike applications in cereals [19].

The fungi-resistance genetic basis to triazoles is not well known. In many cases it seems to be polygenic and observed decreasing effectiveness does not always imply loss of yield performance. The triazol group has many benefits such as high antifungal activity, low toxicity to other organisms, curative properties, and they are compatible with an integrated disease management; however its preventive action is low. That is why they are usually used in mixed formulations with other chemical groups to compensate this deficiency.

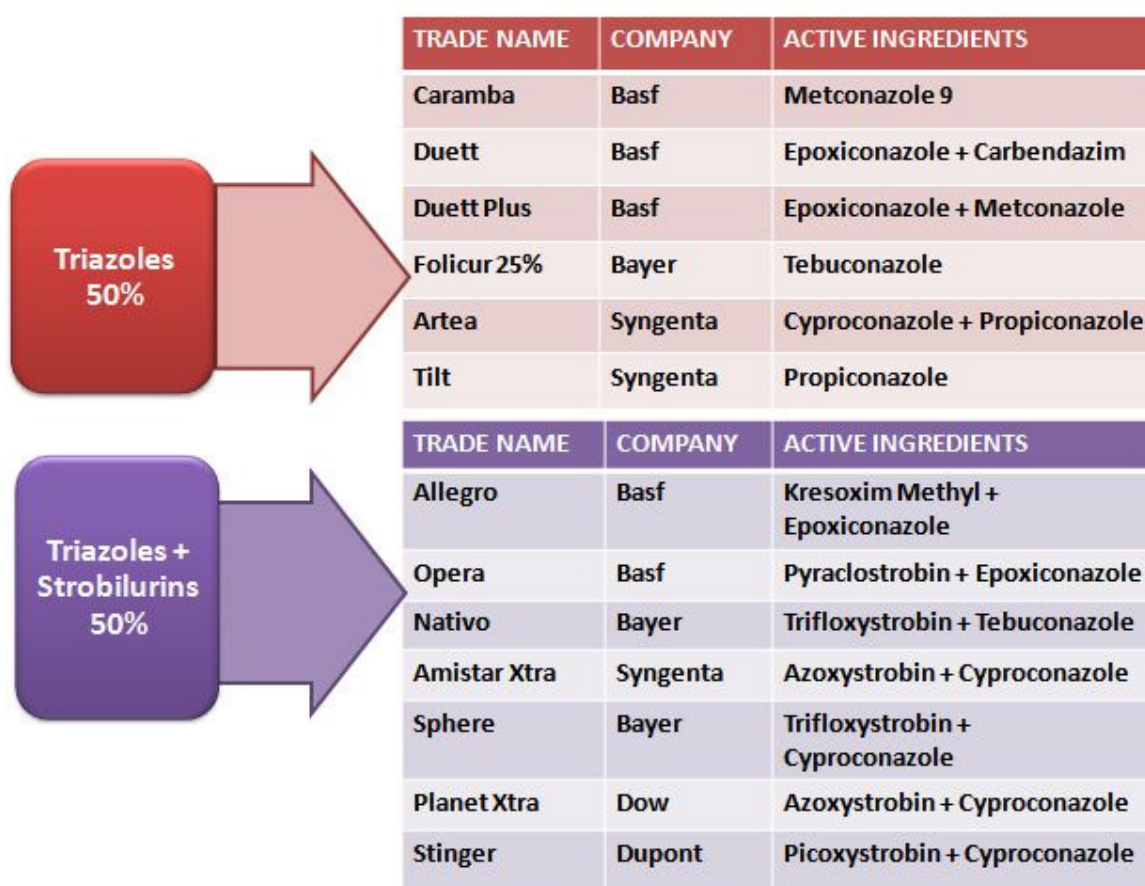


Figure 2. Most common fungicides used in Argentina.

On the other hand, strobilurins are a chemical group which act as mitochondrial respiration inhibitors (MRIs). The strobilurins are an important class of agricultural fungicides, the discovery of which was inspired by a group of natural fungicidal derivatives of β -methoxy-

acrylic acid [20]. Strobilurins are synthetic derivatives of the Basidiomycete fungus *Strobilurus tenacellus*, which grows on pine wood producing decomposition. This chemical group reduces or eliminates competition with other microorganisms that uses wood as a source of food. Strobilurins have become a valuable tool for disease management, as this group controls Oomycetes, Ascomycetes and Basidiomycetes, the three major groups of plant pathogenic fungi in crops. However, strobilurins vary in their levels of activity against the different plant diseases and not all of them give high levels of control of all three major groups of plant pathogenic fungi [20].

Strobilurins mode of action was not considered generating resistance initially; but in recent years resistance to this group has been found in different countries on several diseases, therefore it is essential to achieve an appropriate disease management to avoid these kind of problems [21].

Strobilurins are mesostemic compounds (except Azoxyastrobin which is partially systemic), which means they possess strong adsorption and cuticle-waxes penetration on leaves. Most of the strobilurins are lipophilic and therefore, the active ingredient is moved into the leaf and may enter through the cuticle of the lower leaf surfaces. Consequently, the fungicide may be found on both leaf surfaces even if only one was treated. This movement may take one or a few days and it may move in vapor phase in the air layer adjacent to the leaf surface as well. These processes might be especially important in crops with dense canopy as in the case of wheat in advanced development stage [19]. Moreover, strobilurins are excellent preventive fungicides because they can kill spores. Nevertheless, they are not curative fungicides, since strobilurins binds tightly to the leaf cuticle and therefore the amount of active ingredient present into the leaf tissue would be lower than in the cuticle, being insufficient to control the fungus once it has entered in the plant. Furthermore, the germinative spores are more sensitive to the strobilurins than the mycelium and consequently the best use of the strobilurins is when they are applied before the infection takes place. With this new mode of action the strobilurins are an important addition to the existing fungicide range, particularly for cereals in which recent broad-spectrum fungicide products have been largely based on sterol biosynthesis inhibitors (EBIs) [22]. Therefore, they are generally used in mixtures with triazole fungicides which provides curative power. Finally, strobilurins has an ethylene-synthesis-inhibition-property that cause a delay in leaves senescence and it may causes higher increases in crop yield than other types of fungicides. Wu & von Tiedemann [23] suggested that the fungicide-induced delay of senescence is due to an enhanced antioxidative potential protecting the plant from harmful active oxygen species. A longer period of photosynthetic active green leaf area has been suggested to be the main factor for yield increases obtained with strobilurin fungicides, because the increased photosynthetic period would increase the quantity of assimilate available for grain filling [22].

Strobilurins fungicides have become an integral part of disease-management programs on a wide range of crops in many countries of the world. The major reasons for the success of strobilurins have varied between individual active ingredients, but have consisted of one or more of the following: broad-spectrum activity, control of fungal isolates resistant to other fungicides mode of action, low use-rates and excellent yield and quality [20].

4. The use of fungicides in the integrated foliar disease management to enhance wheat yield and quality

Crop potential yield is defined as that attainable yield, when no nutrient or water limitations occur, i.e. when incident radiation, temperature and physiological crop genotype characteristics determine yield [24]. On the other hand, grain quality has several definitions depending on the users; therefore, the end-use quality is vastly diverse [25]. Several factors have influence on the severity of the main foliar diseases of wheat, among them resistance of the cultivars, tillage systems, N fertilization and fungicide applications.

Genetic resistance is the basis of the integrated disease management. Plant disease resistance can be classified into two categories: qualitative resistance, conferred by a single resistance gene (also termed as race non-specific or slow rusting resistance) and quantitative resistance, mediated by multiple genes or quantitative trait loci (QTLs) (also termed as race non-specific or slow rusting resistance) with each providing a partial increase in resistance [26]. Considering the main foliar diseases in wheat during the last decade, 18 major genes conferring resistance to the pathogen have been identified for resistance to *Septoria tritici*. They were: *Stb1* located on the chromosome 5BL [27], *Stb2* on the chromosome 3BS [28], *Stb3* on the chromosome 6DS [29], *Stb4* on the chromosome 7DS [30]; *Stb5* on the chromosome 7DS [31]; *Stb6* on the chromosome 3AS [32]; *Stb7* on the chromosome 4AL [33]; *Stb8* on the chromosome 7BL [34]; *Stb 9* on the chromosome 2B [35], *Stb10* on the chromosome 1D [36]. *Stb 11* on the chromosome 1BS [37], *Stb12*, on the chromosome 4AL [36], *Stb13* on the chromosome 7BL [38], *Stb14* on the chromosome 3BS [38], *Stb15* on the chromosome 6AS [39], *Stb16* on the chromosome 3D [40], *Stb17* on the chromosome 5A [41] and *Stb 18* on the chromosome 6DS [42]. In addition, several QTL were also found. Eriksen *et al.* [43] found some on chromosomes 2BL, 3AS, 3BL, 6B and 7B. In Argentina resistance was localized in several foreign lines [41]

Considering resistance to tan spot eight races of the pathogen has been characterized based on their ability to cause necrosis and/or chlorosis in differential wheat lines [44]. In Argentina and in general around the world cultivars with acceptable levels of resistance to tan spot and *Septoria* leaf blotch are scarce.

Considering leaf rust, more than sixty genes for leaf rust resistance (*Lr*), most of them major or race specific genes, have been catalogued to date in wheat [45, 46]. However, the gene-for-gene interaction between host resistance genes and pathogen virulence genes combined by virulence shifts in pathogen populations have reduced the effectiveness of a significant number of major leaf rust resistance genes [47, 48]. Replacement of highly variable land races by higher yielding, pure-line varieties in many parts of the world, including the South Cone, has further reduced the wheat gene pool and favored virulence shifts events in pathogen populations.

In Argentina using molecular markers, a set of 66 adapted cultivars previously evaluated by gene postulation for presence of 15 *Lr* genes was screened, and eight genes were detected: six seedling genes (*Lr9*, *Lr10*, *Lr19*, *Lr24*, *Lr26*, *Lr47*) and two adult plant resistance genes (*Lr34*, *Lr37*). Genes *Lr20*, *Lr21*, *Lr25*, *Lr29*, *Lr35* (adult plant resistance gene) and *Lr51* were

not detected in tested cultivars [49]. Resistance in most Argentinean cultivar and around the world is conditioned by one or a few genes.

In the Rolling Pampa region of Argentina, conservation management practices such as no tillage are increasing as alternative cropping systems. No tillage systems have been implemented to restore soil structure in large areas cultivated with double-crop sequences such as wheat (*Triticum aestivum* L.)/soybean (*Glycine max* L. (Merr.); corn (*Zea mays* L.) - wheat/soybean; or wheat monoculture [50]. 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 [51]. 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 [52]. No tillage can also reduce costs by decreasing fuel consumption required to produce a crop [53].

However, in the wheat/soybean system under no tillage, as in wheat following wheat, the inoculum of necrotrophic fungi usually survives until the next wheat season; typically, a minimum of one to two years between wheat crops is required to reduce populations of these organisms [54]. In no tillage systems, crop residue mineralization is slow. It requires 14 to 16 months in Brazil [55] but approximately 18 to 32 months in Argentina and Uruguay due to lower average temperatures than in Brazil [56, 57]. No 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 [58].

Tan spot and Stagonospora blotch [*Phaeosphaeria avenaria* (G.F. Weber) O. Eriksson f. sp. *triticea* T. Johnson, anamorph *Stagonospora avenae* (A. B. Frank) Bissett f. sp. *tritica* T. Johnson] increased in no tillage systems in wheat monoculture or wheat following fallow, although the opposite occurred when wheat followed other crops [59, 60, 61, 62, 63]. In some studies, conventional tillage increases crop residue mineralization, reducing fungal inoculum [61, 64]. However, others [23, 58, 59, 61, 65, 66, 67, 68, 69] reported contrasting results regarding the effect of no tillage on necrotrophic wheat diseases, depending on the environment and the crop growth stage evaluated (early or late in the season).

Fungicides are widely used to manage foliar wheat diseases in Argentina and several countries [70]. The response to fungicide application depends on the severity of specific foliar diseases, cultivar disease resistance or tolerance, management practices, and environmental conditions [71, 72, 73]. Fungicides applied at flag leaf and spike emergence of winter wheat increased mean grain weight and grain yield when they extended canopy life [74]. The green area duration of flag leaf is important because is the last leaf senescing, it intercepts more light than lower leaves and it is in closer vascular proximity to spikes than lower leaves [75]. Strategies to protect flag leaf and delay the senescence process are therefore important to assure not only higher yield but also higher grain quality [76]. Gooding [74] found that the effect of fungicides increasing green area duration of the flag leaf was associated with increases in yield, thousand grain weight and specific weight. Fungicides containing strobilurins to control foliar diseases in wheat are associated in some

cases with higher increases in grain yield and grain weight comparing with triazoles. Dimmock & Gooding [77] reported that strobilurins prolonged green flag leaf area duration and increased mean grain weight significantly more than triazoles.

Jorgensen and Olsen [67] reported wheat yield increases following fungicide treatments ranging from 0.8 to 4.4 Mg ha⁻¹, depending on the amount of infested straw on the soil surface, disease severity and fungicide strategy (type of active ingredient, timing or number or applications, rates and method of application). Severe foliar infections before or at flowering stage of wheat are extremely damaging and may cause important yield losses, whereas when serious infections occur later, the damage to yield is much smaller.

Increased yields disease management are associated mainly with an increase in thousand grain weight [72, 73, 78, 79, 80], while other yield components such as number of spikes.m⁻² [72] or grains.spike⁻¹ [72, 79, 80, 81] are usually not affected by disease severity. However, Simón *et al.* [82] reported that preventing early wheat infection by *Septoria tritici* could result in an increase of spikes.m⁻² and grains.spike⁻¹.

In Argentina, Serrago *et al.* [83] determined that grain number was not affected by foliar diseases when they appeared after anthesis. Grain weight was strongly, poorly or not affected by foliar diseases and was not associated individually with both, the sink size and the source size. However, when the grain weight increment due to fungicide application was plotted against the healthy area absorption per grain, a significant negative association was found for the Argentine experiments [83]. When the healthy absorption area per grain was corrected by the grain weight potential all experiments conducted in Argentine and in France fit well to a common negative linear regression for the relationship between grain weight variation and grain weight potential demonstrating that grain weight potential is an important feature to consider in diseases control programs [83]. Foliar diseases forced the crop to use the accumulated reserves increasing the utilization rate of the water soluble carbohydrates, depleting as a consequence the water soluble content at physiological maturity in all experiments. The association between water soluble carbohydrates and the healthy area absorption per grain corrected by grain weight of healthy crops suggests that foliar diseases in wheat cause source limitation, forcing to the crop to use the water soluble content reserve which could be insufficient to fill the grains previously formed [83].

Management practices such as N fertilization can also affect the expression of wheat foliar diseases [82, 84] and the effectiveness of foliar fungicide application [72, 82, 84, 85]. Increasing N rates may cause negative, positive or neutral effect on foliar disease severity, depending on the geographic location [86] and the type of disease. The magnitude and direction of the influence of N supply on *Septoria* leaf blotch severity has been studied with contrasting results [85, 87, 88, 89]. Simón *et al.* [82, 84, 90] found that in conducive conditions, N fertilization increases the severity of *Septoria* leaf blotch and discussed the effect of different factors affecting the influence of N supply. Increasing N rates retarded tan spot development [66, 69, 73, 91, 92, 93, 94]. However, Bockus and Davis [95] suggested that N applications do not directly affect tan spot severity, but rather appear to reduce disease impact through delayed leaf senescence or that high N rates increase *Septoria* leaf 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 [82, 84, 85, 96, 97]. In addition, experiments carried out in Argentina indicated that yield increase and increase in yield components due to application of tebuconazole was similar in fertilized and non fertilized conditions, despite the increase in the area under disease progress curve under N fertilization [82].

Biotrophic pathogen such as leaf rust also causes important diseases in wheat. N fertilization usually increases the severity of this disease [98, 99, 100].

Using cultivars with good behavior to tan spot, optimizing N rates and fungicide applications would reduce yield losses compared to non fertilized plots planted with susceptible cultivars. Results of some experiments carried out in Argentina addressing this question are presented. Those experiments showed that no tillage often leads to wheat yield losses from diseases caused by necrotrophic foliar pathogens. Conventional tillage reduced foliar disease severity caused mainly by tan spot at GS 23 [101] by 46 and 56% and the area under disease progress curve (AUDPC) [102] by 20 and 14% for each season, respectively compared with no tillage (Table 1). Fungicide and N application reduced disease severity at GS 23 by 35 and 34% respectively, on average over two seasons (Table 1) Disease was less severe in no tillage plots which received a fungicide compared to conventional tillage plots that were not treated with fungicide. Application of 160 kg ha⁻¹ N increased crop biomass by 71% at GS 23 and 57% at GS 83 averaged over two seasons compared to plots that received no nitrogen. N fertilization treatments decreased the AUDPC 17.2% and 23.5%, and fungicide input reduced the disease severity 37.6% and 24.7% in each season. It is remarkable that AUDPC was reduced with N160 as much as with fungicide applications in one of the years (Table 1).

Fungicides increased yield by 9% on average of both years. The increased yield resulted from increases in spikes.m⁻² and thousand grain weight in two seasons, and also from grain.spike⁻¹ in one season [94] (Table 2).

Experiments were also carried out in Argentina with artificial early inoculation with *Septoria tritici* to investigate how N supply influences the disease severity, yield and yield components. In one of the years, with weather conditions conducive to the disease, AUDPC values were higher in the fertilized treatment. In another year with insufficient rain immediately after inoculation, the disease only progressed faster under N fertilization in the flag leaf, which was exposed to conducive environmental conditions from its appearance. The effect of N fertilization was influenced by the cultivar characteristics, climatic, and agronomic conditions (Table 3). Knowledge that N fertilization promotes the development of *Septoria tritici* blotch in conducive conditions will be useful for deciding management strategies of the cultivars and for optimizing conditions for the selection in breeding programmes. Considering yield and yield components, additional N increased yield, spikes.m⁻² and grains.spike⁻¹, but not thousand kernel weight or test weight. The percentage reduction in yield, yield components and test weight due to inoculation was similar in fertilized and non-fertilized conditions, despite the increase in the AUDPC values by N fertilization (Table 4).

	Year 1								Year 2							
	Conventional tillage				No tillage				Conventional tillage				No tillage			
	0N	80N	160N	Average	0N	80N	160N	Average	0N	80N	160N	Average	0N	80N	160N	Average
----- 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

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

LSD (P=0.05) for significant interactions: LSD interaction T x F severity GS 23, 2002=5.82

Table 1. Means for the interactions of cultural practices on foliar disease intensity and wheat biomass over two season at Los Hornos, La Plata, Argentina

	Year 1								Year 2							
	Conventional tillage				No tillage				Conventional tillage				No tillage			
	0N	80N	160N	Average	0N	80N	160N	Average	0N	80N	160N	Average	0N	80N	160N	Average
	-----Yield (kg.ha ⁻¹)-----															
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 (n°)-----															
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 (n°)-----															
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, SPM2 TxN, 2002=74, 2003=96, KPS: LSD T × F, 2002= 3.8; C × N, 2003= 8.4

TKW: LSD T × N, 2002=2.7; C × N, 2002=2.8

Yield: LSD T × N, 2003= 1742

Table 2. Means for the interactions of cultural practices on yield and yield components of wheat over two seasons, at Los Hornos, La Plata, Argentina

Cultivar	Year 1			Year 2		
	With fertilizer	Without fertilizer	Average	With fertilizer	Without fertilizer	Average
Buck Ombú	723 a ^y (812) ^x	634 a (702)	679 ^z E (757)	343 a (351)	370 a (380)	356 B (365)
Don Ernesto	428 a (459)	237 b (273)	332 B (366)	362 a (370)	286 a (295)	324 AB (333)
Klein Centauro	505 a (330)	466 a (272)	486 C (301)	420 a (489)	374 a (445)	397 B (467)
Klein Dragón	265 a (231)	85.3 b (96)	175 A (163)	222 a (246)	258 a (268)	240 A (257)
PROINTA	313 a (343)	169 b (205)	241 A (274)	382 a (391)	332 a (342)	357 B (366)
Federal PROINTA Verde	721 a (778)	423 b (472)	572 D (625)	406 a (292)	336 a (225)	371 B (258)
Averages	492 a	336 b		357 a	326 a	

Means are adjusted by heading date as a covariant.

^x Unadjusted values. ^y Means followed by the same letter in the same row within the same year are not significantly different, LSD (P=0.05). ^z Means followed by the same letter in the average columns within the same year are not significantly different, LSD (P=0.05).

Table 3. Means of the AUDPC of *Septoria tritici* blotch on six wheat cultivars under two nitrogen fertilisation treatments in two years.

Cultivar	Year 1				Year 2				Average 1996	Average 1997
	With fertilization		Without fertilization		With fertilization		Without fertilization			
	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation		
	Kg.ha ⁻¹									
Buck Ombú	5305 (38.1) [†]	8579	4501 (44.9)	8166	5097 (31.3)	7423	4822 (30.6)	6951	6638	6074
Don Ernesto	6521 (26.3)	8852	4949 (32.0)	7251	5157 (27.0)	7062	4176 (29.5)	5925	6888	5580
Klein Centauro	6835 (18.6)	8400	5836 (20.8)	7371	7413 (19.5)	9213	5961 (22.0)	7644	7111	7558
Klein Dragón	9325 (16.6)	11175	6974 (17.7)	8474	6512 (20.8)	8223	5798 (23.5)	7508	8987	7029
PROINTA	6524 (25.3)	8744	4713 (31.6)	6888	5035 (28.2)	7015	4760 (27.0)	6525	6717	5834
Federal PROINTA	6550 (31.4)	9542	5252 (31.5)	7661	4950 (28.0)	6879	4550 (28.0)	6321	7251	5675
Isla Verde	6843 (25.7)	9215	5367 (29.7)	7635	5694 (25.4)	7636	5011 (26.6)	6824	7265	6291
Average Cultivar										
Average fertilization										
With	8029				6665					
Without	6501				5918					
Average inoculation										
With	6185				5353					
Without	8425				7230					
LSD cultivars	249.0				906.8					
LSD fertilization	522.9				598.6					
LSD inoculation	721.0				306.6					

[†] Percentage of reduction relative to the non-inoculated control are given in parenthesis.

Table 4. Means of yield per hectare for six wheat cultivars under two nitrogen fertilization conditions and two inoculation treatments with *Septoria tritici*.

Further experiments were also carried out in Argentina comparing the effect of N fertilization and fungicides on the severity caused by tan spot, *Septoria* leaf blotch and leaf rust and on the yield of wheat in the same environment [103]. Results indicated that there was a three way interaction pathogen \times N fertilization \times fungicide. This interaction was caused by the fact that tan spot severity decreases with N fertilization, but increases for *Septoria* leaf blotch and leaf rust (Fig. 3, 5, 7). The application of N fertilization did not reduce severity of tan spot as much as fungicide application. Fungicides (Nativo: combination of triazoles and strobilurins) were effective in controlling the three foliar diseases, but mainly leaf rust. In addition the control produced by the fungicide was higher when the severity increases. With similar severity values, the control produced by the fungicides was similar for all N treatments. Yield was increased by fungicide application 20% and by N fertilization by 27.5% when the pathogen inoculated was *Septoria tritici* (Fig. 4) and by 10.3% and 18.6% when the pathogen inoculated was *Drechslera tritici-repentis* (Fig. 6) On the contrary, when the pathogen inoculated was *Puccinia triticina*, fungicides caused the higher increase in yield (19.2%), whereas the increase due to N fertilization was 9.2% (Fig. 8).

Grain quality in wheat is a complex of different traits deeply influenced by genotypic and environmental factors. The baking market requires flour for different types of products, e.g. mechanized bread, artisan bread, baguette, flat breads, steamed bread, biscuits, crackers, pasta, noodles, etc. Although varieties are assigned to quality groups when they are registered to be commercialized, the final product after growing and harvesting is not always adequately classified for commercialization.

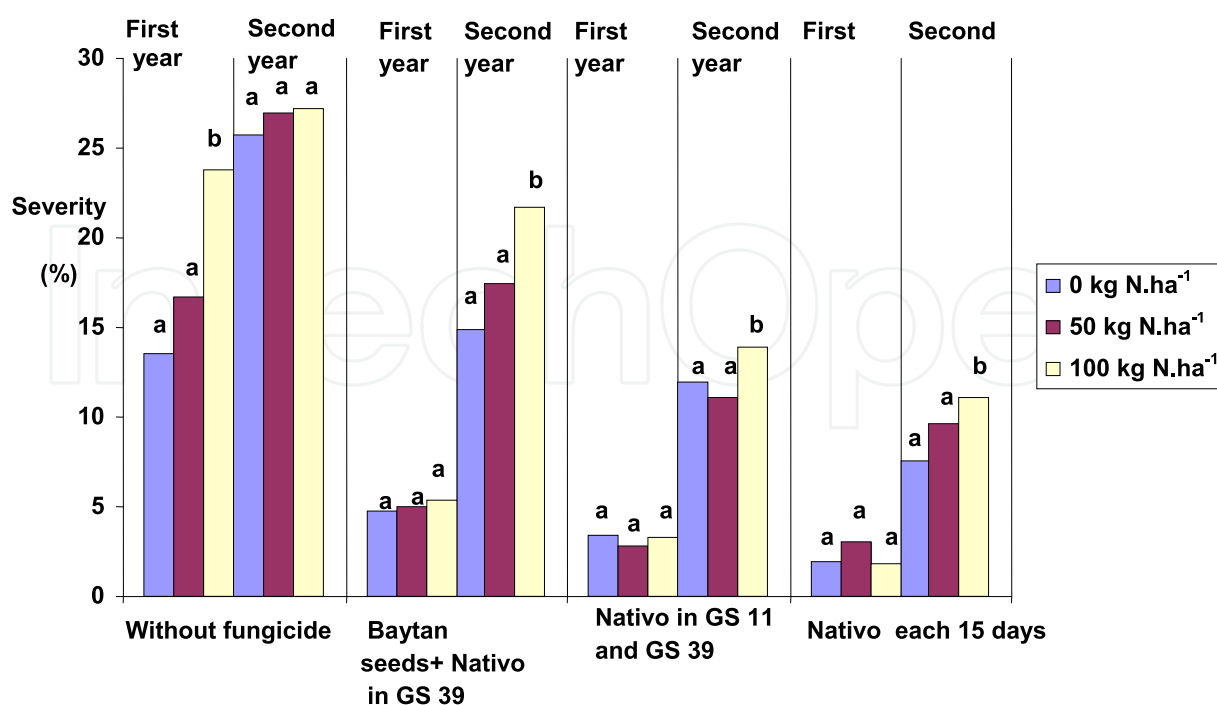


Figure 3. Means of fungicide \times fertilizer interaction of disease severity (%) on a trial inoculated with *Septoria tritici* with three nitrogen levels, four fungicide treatments and two cultivars in two years.

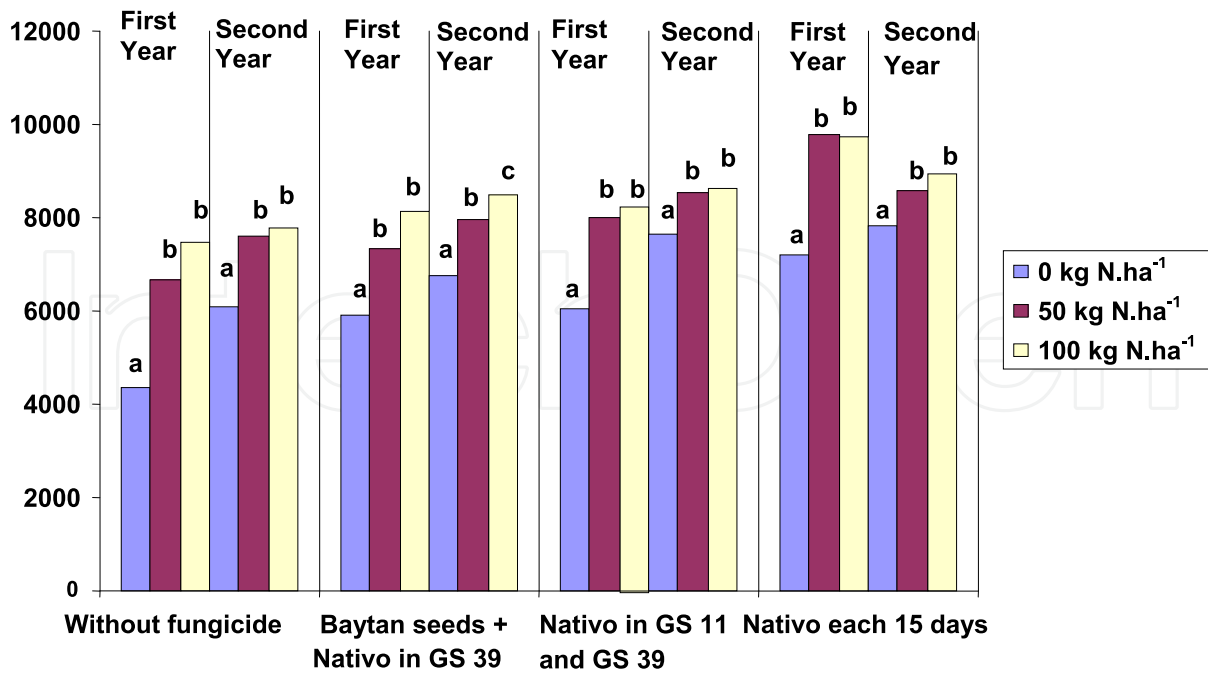


Figure 4. Means of fungicide x fertilizer interaction of grain yield in wheat ($\text{kg}\cdot\text{ha}^{-1}$) on a trial inoculated with *Septoria tritici* with three nitrogen levels, four fungicide treatments and two cultivars in two years.

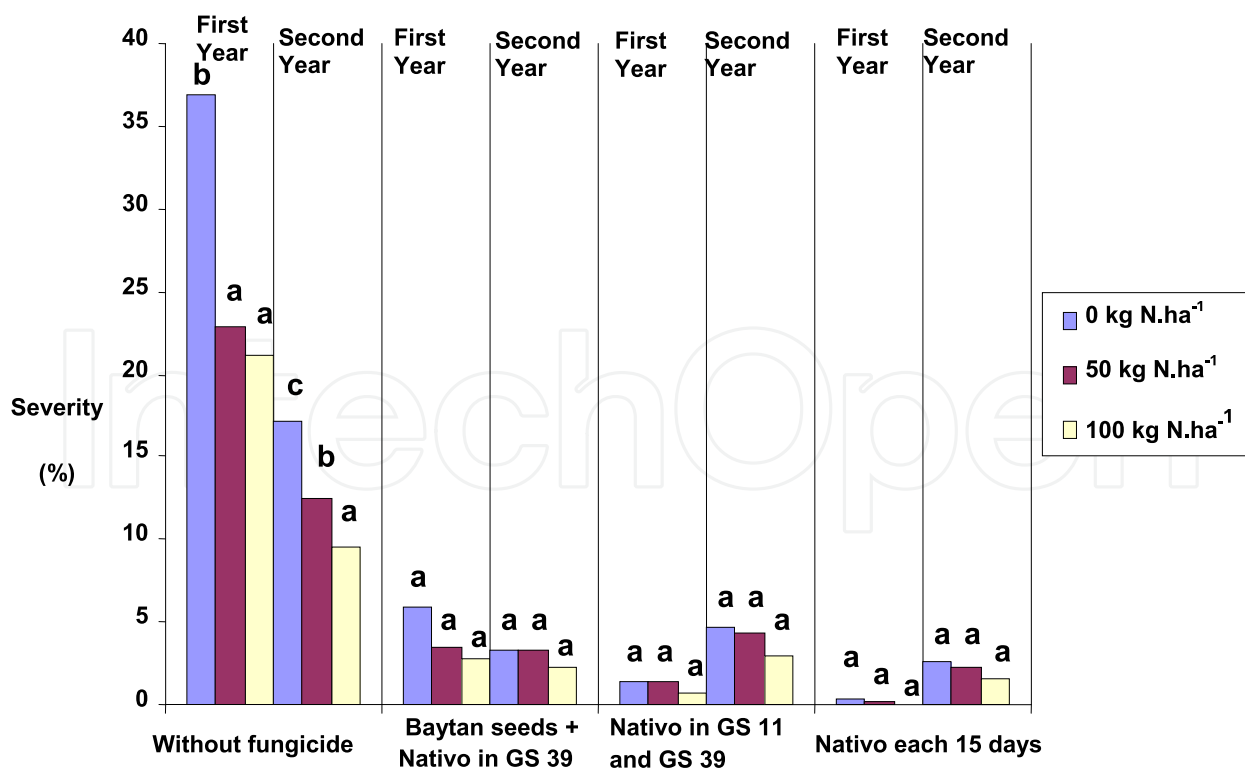


Figure 5. Means of fungicide x fertilizer interaction of disease severity (%) caused by *Drechslera tritici-repentis* in GS 82 on a trial with three nitrogen levels, four fungicide treatments and two wheat cultivars in two years.

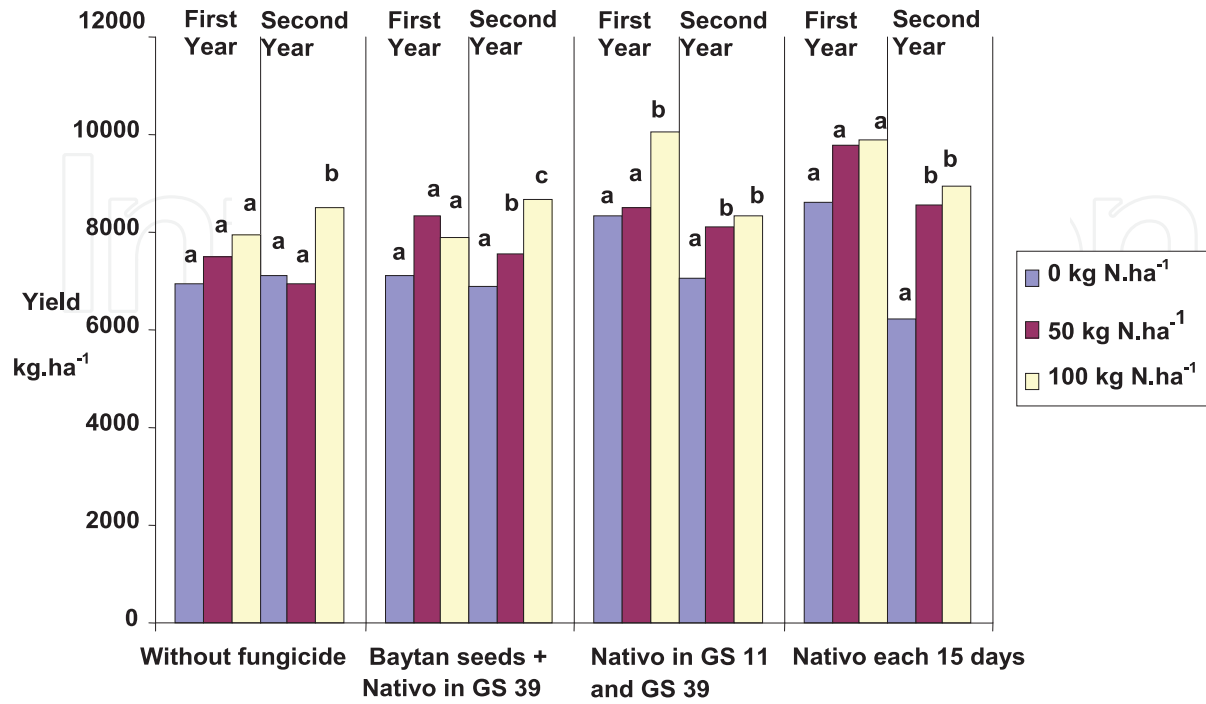


Figure 6. Means of fungicide x fertilizer interaction of grain yield in wheat (kg.ha⁻¹) on a trial inoculated with *Drechslera tritici-repentis* with three nitrogen levels, four fungicide treatments and two cultivars in two years.

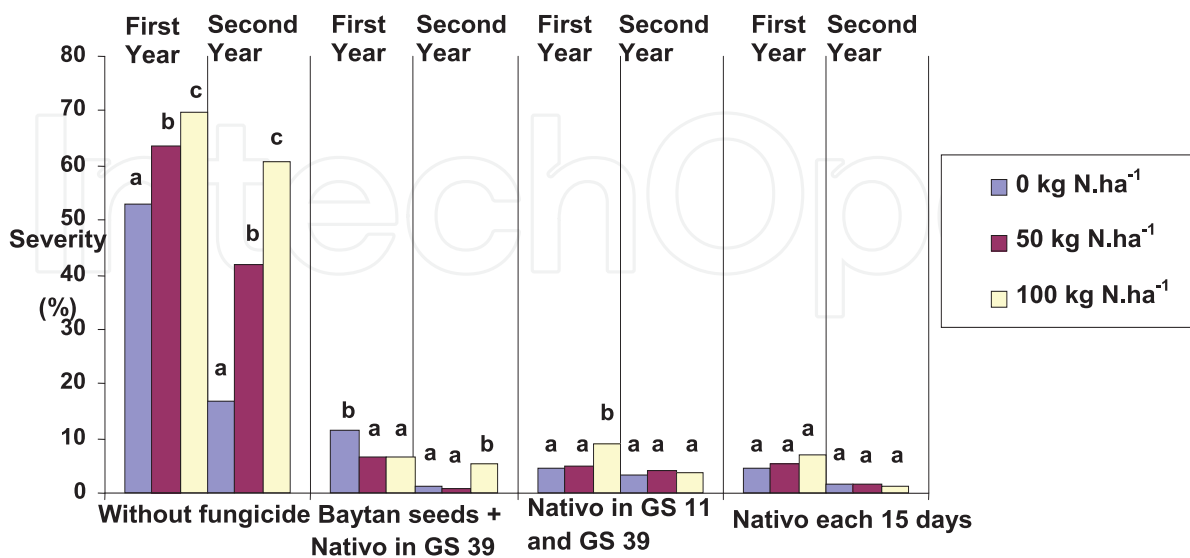


Figure 7. Means of fungicide x fertilizer interaction of disease severity (%) caused by *Puccinia triticina* in GS 82 on a trial with three nitrogen levels, four fungicide treatments and two wheat cultivars in two years.

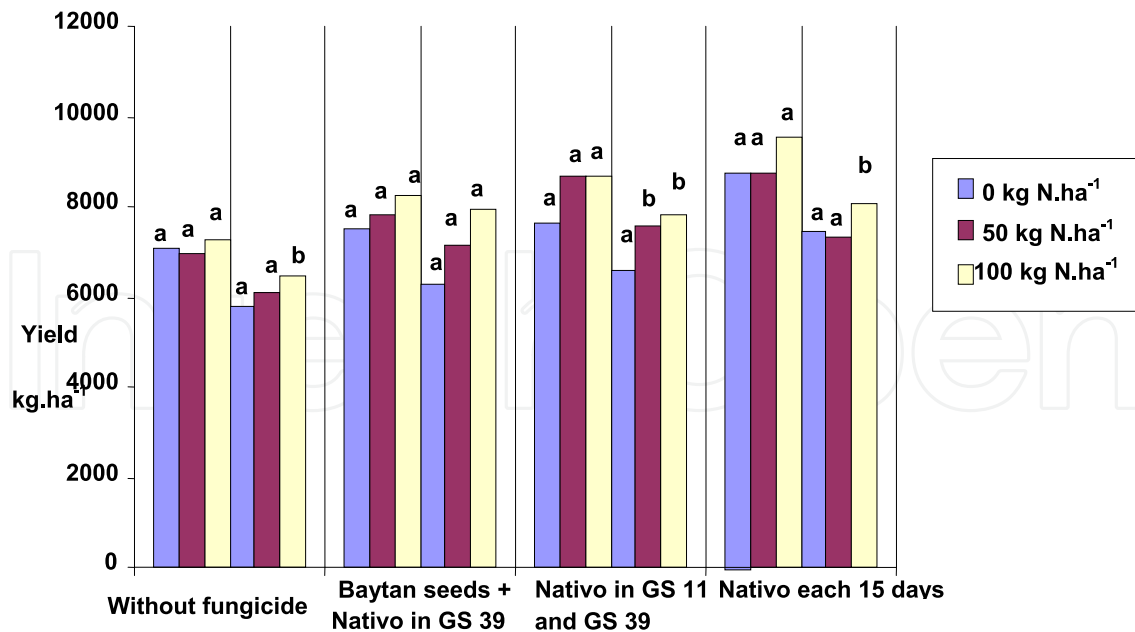


Figure 8. Means of fungicide x fertilizer interaction of grain yield in wheat (kg.ha⁻¹) on a trial inoculated with *Puccinia triticina* with three nitrogen levels, four fungicide treatments and two cultivars in two years.

The main quality characteristics for the wheat utilization are flour extraction (milling yield), flour protein concentration and rheological-breadmaking properties. The behavior of dough is strongly linked to the type and amount of protein present in flour, and hence the concentration of protein in the wheat grain at harvest. Grain protein concentration is positively associated with breadmaking quality, particularly to loaf volume [104]. In most production systems there is a negative relationship between yield and grain protein concentration. Nevertheless, this does not imply that higher grain protein cannot be obtained at high-yield levels. At low N rates of fertilization (Fig. 9), yield increases asymptotically, i.e. the response of starch accumulation is greater than protein content (zone 1) [105]. The first increments of N tend to increase yield but decrease protein percentage, resulting in the frequently reported negative relationship between grain yield and protein percentage (zone 1). After a certain level of N is attained, the response of starch and protein accumulation has a different response (zone 2). At these N fertilization levels, additional N results in a lower yield increase regarding the previous N doses (but still positive), and a comparatively higher increase in protein percentage. Finally, with higher amounts of N, the crop reaches a third region of response (zone 3), where maximum yield may be attained. At this point, additional fertilizer does not affect the amount of starch in the grain, but increases protein content (Fig. 9). On the other hand, different genotypes generate different protein concentrations in grain, depending on N rates fertilization and how efficiently they absorb and use N for yield generation. The increase in grain protein content under high N fertilization conditions results in greater synthesis and accumulation of storage protein (gliadins and glutenins), which are the gluten forming proteins [106]. Gluten proteins are the major determinant of the processing properties of wheat dough, by conferring viscoelasticity, which is essential for breadmaking process.

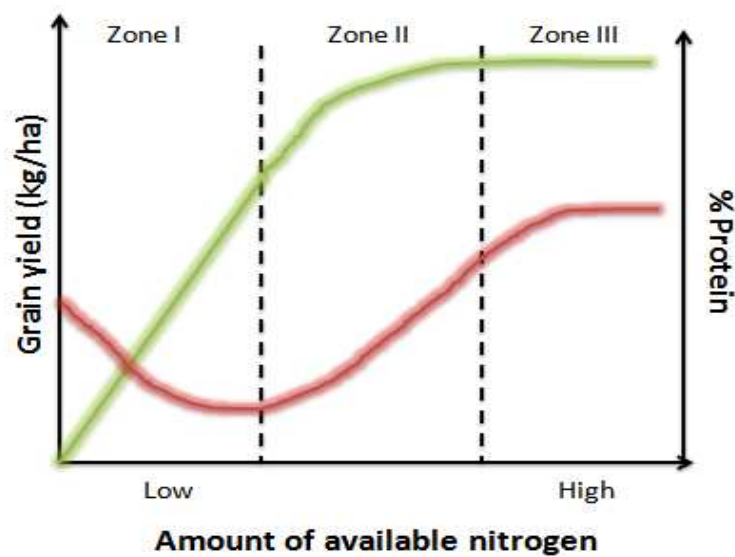


Figure 9. Diagrammatic representation of the response of yield and protein percentage to nitrogen fertilizer [105].

Little attention has been given to foliar diseases impact on milling and baking quality and to the interactions of disease severity \times cultivar on the wheat quality. These effects are more significant when strobilurins are applied due to the prolongation of the green flag leaf area duration compared with triazoles. Flag leaf photosynthesis in wheat contributes about 30-50% for grain filling [77], and longevity of the flag leaf promoted by strobilurins affects concentration of protein in the grain.

Gooding [74] reported fungicide effects on crude protein concentration depending on cultivar and disease control. The effect of foliar diseases on protein content may vary depending on foliar disease type. When biotrophic fungal pathogens such as leaf rust affects wheat, the protein concentration usually decreases, (i.e. the pathogen causes more damage on the accumulation and partitioning of N in the grain than on the accumulation and partitioning of dry matter) leading to a modification of the rheological properties of flour [74, 79, 107]. On the other hand, when wheat is affected by necrotrophic pathogens as tan spot, protein concentration increases [108]. Finally, hemibiotrophic pathogens such *Septoria* leaf blotch may cause both effects, depending on the genotype and environmental conditions. Controlling *Septoria* leaf blotch usually reduced protein concentration [79]. Liaudat [109] found increases in protein concentration when severity of *Septoria* leaf blotch increases. In the same study, the disease control with fungicide produced decreases in protein concentration and this reduction was more significant when strobilurins were applied.

5. The effect of fungicides on mycorrhizae

Arbuscular mycorrhizal fungi (AMF), which form symbiotic associations with root systems of most agricultural species, have been suggested as widespread potential bioprotective agents, inducing local and systemic resistance to some diseases. The knowledge of these fungi populations could also be an interesting contribution for the integrated disease management. Arbuscular mycorrhizae are associations between fungi that belong to the

phylum Glomeromycota [110] and most plant species [111]. Whereas there are numerous studies on the biocontrol effect of arbuscular mycorrhizae, there are relatively few on the effects of fungicides on these beneficial associations.

Arbuscular mycorrhizae are considered beneficial to plants, although their positive effects are variable because mycorrhizal symbioses reflect complex interactions among the plant, the fungi, and the environment [112, 113]. In agriculture, research dealing with mycorrhizal fungi is valuable both for determining appropriate management strategies and as a background to achieve successful inoculations [114]. The interaction between the fungus and its host plant mainly consists of nutrient transfer (the plant provides the arbuscular mycorrhizal fungi with photosynthates while the fungus delivers nutrients to the plant). The increased nutrient uptake from the soil, particularly of phosphorus and nitrogen, is the main benefit attributed to mycorrhizal symbiosis [115, 116]. However, other benefits are enhancement of resistance to root parasites [117], improvement of drought tolerance [118] and mitigation of environmental stresses such as salinity [119]. Another important role attributed to arbuscular mycorrhizal fungi is improving soil stability, which may diminish erosion [120, 121, 122, 123]. Recent studies have found evidences of bioprotective effect of arbuscular mycorrhizal fungi against fungal pathogen, mainly those causing soil-borne diseases [124, 125, 126]. Arbuscular mycorrhizal fungi may control plant pathogens or contribute to activate plant defence responses through direct or indirect mechanisms, such as: improving plant nutrition and damage compensation [115], anatomical alterations in the root system [127], microbial changes in the rhizosphere and enhancing the attenuated plant defence responses by altering the host's signalling pathways [128]. Nevertheless, the knowledge about the induction of plant defence responses, the genetic, biochemical and signalling factors, their mechanisms and pathways involved, is still low [129].

The studies related to the effect of arbuscular mycorrhizal fungi on reduction of root diseases produced by fungi have mainly focused on those rots produced by species of *Phythium*, *Phytophthora*, *Fusarium*, *Verticillium*, *Pyrenochaeta*, *Gaeumannomyces*, *Sclerotium*, and *Rhizoctonia* [130]. Regarding foliar diseases, Gernns *et al.* [131] reported a compensation mechanisms between mycorrhizal plants and biotrophic fungal diseases. They found that mycorrhizal barley-plants were more susceptible to the obligate biotrophic shoot pathogen *Erysiphe graminis* f. sp. *hordei*, however, mycorrhizal plants suffered less than non-mycorrhizal plants in terms of grain number, spikes yield and thousand-grain weight. As mentioned before, other bioprotective effect of arbuscular mycorrhizal fungi on wheat is that found against take-all disease caused by *Gaeumannomyces graminis* [132, 133].

On the other hand, little is known about the effect of fungicides on mycorrhizal colonization, sporulation or spore germination. The effect of fungicide on arbuscular mycorrhizal fungi may be direct on the fungal growth or indirect, through changes in the physiology of the host plant, reductions in the disease levels and/or modifications in the soil environment. Considering the fungal component of mycorrhizal plants, is reasonable to infer that some fungicides might affect mycorrhizal colonization. Fungicides comprise a huge variety of compounds that differ in their effect on the host physiology, mode of action, spectrum of activity, application methods and formulation. Several studies have shown that fungicides

can affect mycorrhizal associations in a negative, neutral or even in a positive manner [134]. Consequently, it is difficult to generalize about the effects of fungicides on arbuscular mycorrhizal fungi. It is fundamentally important to distinguish the foliage fungicide applications, to those which are directed to the soil, or those which are applied on seeds.

In field crops, in the Pampas region, the application of fungicides to the soil is not usual. However the so-called "seed treatment" make contact with soil, and then, direct effects of fungicides on the external hyphae and / or spores impacting the functionality of the symbiosis are expected. Thiram is one of the classic fungicides used for seed treatments, with preventive and contact action, belonging to the dithiocarbamate group. Inhibitory effects on root colonization and spore production of dithiocarbamates applied as soil or seed treatments have been widely reported in the literature [135, 136, 137, 138]. Among the triazole compounds, triadimenol is widely used for seed treatments in wheat. Triazoles act as inhibitors on the biosynthesis of ergosterol, a major component of fungal membranes. Since the relative amount of ergosterol in the Glomeromycota is low compared to other groups of fungi, the negative effect of triazole application on arbuscular mycorrhizal fungi is generally low [139, 140]. The active ingredient metalaxyl is a widely used systemic seed treatment used for different crops. It has been found that metalaxyl applications increased mycorrhizal colonization and plant growth [141, 142]. This fungicide is specific controlling plant pathogenic oomycetes, and has no effects on other groups of fungi. Therefore, it has been suggested that its favorable effect on mycorrhizal colonization is primarily indirect, through reductions in populations of antagonistic organisms to arbuscular mycorrhizal fungi [143]. However, Giovannetti *et al.* [137] documented direct effects of this fungicide, since the application of metalaxyl stimulated spore germination and hyphal growth in the pre-symbiotic phase of Glomeromycota *in vitro*. Although these studies show interesting trends, conditions of sterile culture media are markedly different to those occurring in field soil, because of a large number of factors, including fungicide absorption by the soil. Within the classical fungicides for seed treatment, which are being gradually replaced by modern ones, there are those belonging to the group of benzimidazoles such as benomyl and carbendazim. Benomyl and other benzimidazoles decompose to methyl benzimidazole carbamate (carbendazim), and the latter compound interferes with the division of the nuclei of sensitive fungi. The deleterious effect of benomyl or carbendazim (the latter still used in seed treatment) on the arbuscular mycorrhizal fungi is widely known. Benzimidazoles specifically bind to beta-tubulin, thereby inhibiting the tubulin function, which is crucial for fungal growth [144, 145, 146, 147, 148]. Venedikian *et al.* [149] found that mycorrhizal colonization may be less inhibited by carbendazim applications than spore germination and hyphal growth in agar medium. This suggests that different growth phases of these fungi can tolerate different fungicide concentrations [150, 151, 152].

Regarding fungicide foliar applications, negative effects of triazole at high doses or repeated applications on mycorrhizal colonization have been reported [153, 154]. However, in a wheat crop in Argentina, Schalamuk *et al.*, 2011 (unpublished) found that triazole applications did not reduce mycorrhizal colonization. When considering the evaluation of the effects of foliar fungicides on arbuscular mycorrhizal fungi it should be taken into

account not only the effect of the compound *per se*, but also the reduction in disease generated by increasing green leaf area and photosynthate supply to the roots. On the other hand, the strobilurins group, with mesostemic and trans-laminar action, is rapidly spreading in the Argentinean agricultural region. Fungicides of this group possess a broad-spectrum action, inhibiting mitochondrial respiration. Diedhiou *et al.* [154] found that strobilurins, despite its broad spectrum, did not negatively affect mycorrhizal colonization of crops when applied to control foliar pathogens at recommended doses. Schalamuk *et al.* [155] found similar results in wheat. Since the mode of action of this group of foliar fungicides is not fully systemic, it is questionable if strobilurin applications would present a detrimental effect on arbuscular mycorrhizal fungi.

Concerning the effect of fungicide application on the diversity of Glomeromycota, the information on this topic is low, although it is recognized that there are differences in sensitivity to fungicides among different groups or isolates among Glomeromycota taxa [150].

6. Conclusions

The grain production region has experimented severe tillage changes in the past twenty years in Argentina, mostly due to the increased interest in maintaining soils covered with plant residues and the increase used of N fertilization necessary to achieve high yield and grain quality.

In the wheat/soybean system under no tillage, as in wheat following wheat, the inoculum of necrotrophic fungi usually survives until the next wheat season. Therefore, the use of fungicides is essential to decrease the severity of necrotrophic diseases.

The results of experiments carried out in Argentina indicates that sowing wheat following wheat in no tillage is possible without significant yield losses if effective disease management practices including moderately resistant cultivars, N fertilization and fungicides are applied.

N fertilization increases the severity caused by leaf rust whereas decreases the severity caused by tan spot

Increased yields by disease management are associated mainly with an increase in thousand grain weight while other yield components such as number of spikes.m⁻² or grains.spike⁻¹ are usually not affected by disease severity. However, preventing early wheat infection by *Septoria tritici* could result in an increase of spikes.m⁻² and grains.spike⁻¹.

Some studies determined that grain number was not affected by foliar diseases when they appeared after anthesis. Grain weight was strongly, poorly or not affected by foliar diseases and was not associated individually with both, the sink size and the source size. However, when the grain weight response due to fungicide application was plotted against the healthy area absorption per grain, a significant negative association was found for the Argentine experiments.

Further experiments carried out in Argentina with wheat cultivars inoculated with the causal agent of tan spot or Septoria leaf blotch or leaf rust determined that there was an interaction pathogen \times N fertilization \times fungicide. This interaction was caused by the fact that tan spot severity decreases with N fertilization, but increases for Septoria leaf blotch and leaf rust. Fungicides (combination of triazoles and strobilurins) were effective in controlling the three foliar diseases, but mainly leaf rust. In addition the control produced by the fungicide was higher when the severity increases.

It is difficult to generalize about the effects of fungicides on arbuscular mycorrhizal fungi, because they may have positive, negative or neutral effects. In a wheat crop in Argentina it was found that neither triazole nor strobilurins applications reduce mycorrhizal colonization.

Further studies should be done with different cultivars to determine the effect of tolerance and its control mechanisms, in addition to N fertilization and fungicide applications on yield and quality when wheat is affected by necrotrophic or biotrophic pathogens. Furthermore, field experiments on the effect of fungicides on mycorrhizal fungi in wheat in Argentina are recent and should be intensified.

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