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Italian ryegrass (*Lolium multiflorum* Lam.) density and N fertilization on wheat (*Triticum aestivum* L.) yield in Argentina

J.A. Scursoni*, Mauro Palmano, Agustín De Notta, Diego Delfino

Facultad de Agronomía, Cátedra de Producción Vegetal, FAUBA, Av. San Martín 4453 (1417), Buenos Aires, Argentina

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ABSTRACT

Italian ryegrass (*Lolium multiflorum* Lam.) is an important and troublesome weed in cereal crops in Argentina. It is present in 46% of the wheat fields of the most important cropped area and its control is mainly performed by selective grass herbicides that represent a significant percentage of total production cost. Designing weed management strategies is necessary to reduce the dependence on herbicides. This requires improving our knowledge of the effect of different management practices such as N fertilization on weed-crop interactions. Given the importance of achieving a sustainable and efficient use of herbicides, field experiments were carried out at wheat field crops in Argentina between 2004 and 2006. The main objective was to evaluate the competitive effect of ryegrass at different densities on wheat yield and its components with different nitrogen fertilization levels. In addition, the effect of N fertilization on weed emergence, growth and fecundity was also studied. N fertilization increased ryegrass competitiveness. Ryegrass densities of 100 plants m⁻² reduced wheat yield by 30% and 20% with and without N fertilization, respectively. N fertilization did not modify seedling emergence dynamics but increased ryegrass growth and fecundity. Individual fecundity was 190 and 118 seeds per plant on the average of fertilized and non-fertilized plots, respectively.

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1. Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) is a troublesome annual grass weed influencing the productivity of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) crops worldwide (Holm et al., 1977; González-Andujar and Saavedra, 2003; Trusler et al., 2007; Paynter and Hills, 2009). In Argentina, wheat sown area in 2010–011 was 4,400,000 ha and 30% of this was carried out in the south-east of Buenos Aires Province (Minist. Agric., Ganad. y Pesca, Argentina, 2011), between 37°–39° S, and 58°–61° W. Scursoni et al. (2007) reported Italian ryegrass in 46% of wheat fields surveyed in that area.

In Argentina, ryegrass control is mainly achieved by applying herbicides such as diclofop methyl, clodinafop propargyl, pinoxaden and iodosulfurón plus metsulfuron methyl. The application of selective grass herbicides represents a significant proportion of total production costs. In addition, there are many studies showing resistance evolution of ryegrass species to these herbicides (Kuk et al., 2000; Ellis et al., 2010). *Lolium* spp. has evolved resistance to diclofop methyl and other ACCase inhibitors in 15 countries (Valverde and Heap, 2009). Currently there are 27 biotypes of Italian ryegrass resistant to ACCase inhibitors around the world (Heap, 2011).

While herbicides are a main component of weed management, other agronomic practices such as seeding rates, competitive crops, strategic fertilizer placement, narrow row spacing can also be used to suppress and manage weeds in an integrated weed management system, reducing dependence upon herbicides (Paynter and Hills, 2009). González-Andujar and Fernández-Quintanilla (2004), modeling the population dynamics of *Lolium rigidum* soil seed bank in different barley crop systems, found that keeping the prevailing crop rotation (continuous barley) and integrating the use of various chemical and cultural tactics (delayed seeding, crop competition, and preventing dispersal of seeds before harvest) resulted in better long-term results compared to a standard system based mainly on herbicide application.

The development of weed management strategies requires improving our knowledge of weed-crop interactions. Farmers are becoming increasingly interested in more comprehensive weed management programs which would lessen weed populations over time, thus reducing their dependence on herbicides (Liebman et al., 2001). Management practices that increase the competitive ability





^{*} Corresponding author. Tel./fax: +54 4524 8025. E-mail address: scursoni@agro.uba.ar (J.A. Scursoni).

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of crops with weeds can be important components of integrated weed management systems (Blackshaw and Brandt, 2008). Fertilization is an important agronomic strategy commonly used to increase crop yield. Although nutrients promote crop growth, many studies have shown that, in some cases, fertilizers benefit weeds more than crops (Di Tomaso, 1995).

Nitrogen is the major nutrient added to increase wheat yield, but it is not always known how N levels can affect weed demographic processes and crop—weed competitive interactions (Blackshaw et al., 2003). The knowledge of the relationship between wheat yield and ryegrass densities is a useful tool to evaluate the economic threshold and the benefit of herbicide application. However, there are few studies in Argentina focused on competitive effects of ryegrass or on the effect of nitrogen fertilization on wheat-ryegrass competition.

Given the importance of achieving a sustainable and efficient use of herbicides, this study was carried out with the main objective of evaluating the competitive effect of ryegrass populations at different densities ranged from 10 to 350 plants/m², on wheat yield and its components at different nitrogen fertilization levels. In addition the effect of N fertilization on ryegrass growth and fecundity was studied.

2. Materials and methods

2.1. Site descriptions and experimental design

Two field experiments were conducted during 2004–05 and 2005-06 on farmers' production fields infested with Italian ryegrass in Azul (36° 47' S and 59° 51' W) in Buenos Aires province and in Marcos Juarez (32° 42′ S, 62° 06′ W) in Cordoba province, respectively. An additional field experiment was carried out at the Experimental Station of the Faculty of Agronomy, Buenos Aires (34° 36' S, 58° 22' W) in 2005/06. Soils at experimental area were Typic argiudoll. In order to perform soil analysis, six soils samples (0–20 cm) were taken in each experimental area. Results were 2.3%-2.6% and 2.8% OM, 38-38.7 and $40 \text{ kg N} \text{ ha}^{-1}$ and pH 5.9-6.1and 6.2, for Azul, Marcos Juarez and Buenos Aires, respectively. Annual rainfall in each experimental year was 824 mm, 760 mm and 1088 mm in Azul, Córdoba and Buenos Aires, respectively. Wheat was sown to achieve a density of 300 plants m^{-2} in a no-till system in rows 15-cm apart, at a depth of 3 cm on 13 July, 24 June and 28 July in Azul, Marcos Juarez and Buenos Aires, respectively. Crop density was checked at 12 Zadoks growth stage (Zadoks et al., 1974). At all experimental sites the entire area was fertilized before sowing with band-placed 21.6 kg N ha⁻¹ and 55 kg P ha⁻¹.

Completely randomized design experiments were installed in Azul and Buenos Aires. Sixty two permanent quadrats (0.2 m^2) were established on a wheat cropped area of 0.5 ha. Forty eight quadrats were established with ryegrass densities ranging from 10 to 350 plants m⁻²; eight quadrats with wheat without ryegrass and other six only with ryegrass at three densities, 100, 200 and 300 plants m⁻². Half of the total number of quadrats were fertilized by broadcasting 110 kg N ha⁻¹ at the onset of crop tillering. Ryegrass emergence and density were assessed periodically and plants were thinned in order to achieve a constant ryegrass density in each plot. There were few other weeds in the experimental area so they were thinned by hand.

In Marcos Juarez, two different wheat cultivars (Buck Gaucho and Nidera Baguette 13) were sown with the objective of evaluating their competitiveness. These cultivars were selected because they are very representative of that area. The experimental design was a split plot with four replications for each treatment (cultivar \times ryegrass density). Main plot was wheat cultivars and the subplots were different ryegrass densities: 0, 35, 70, 105, 140, 175,

210, 245 and 280 plants m^{-2} . In each plot of 6 m^2 , quadrats of 0.5 m^2 were established, ryegrass seedling density was assessed periodically and individuals were thinned to keep ryegrass density constant. All the plots were fertilized with 125 kg N ha⁻¹ at the beginning of crop tillering.

2.2. Data collection

Wheat and ryegrass emergence were determined in each quadrat. Ryegrass seedlings were identified during the crop cycle with color plastic cable rings in order to identify different seedling cohorts. At crop maturity, ryegrass plants were removed by hand, spikes were counted and fecundity was assessed from a sample of 50% of the spikes. After that, plants were dried for 48 h at 60 °C. Wheat plants were also removed from each quadrat at maturity. Then, spikes were counted and a sample of thirty percent of the whole spikes was threshed to attain the yield per quadrat. Then, one thousand grain weight was assessed and the total number of grains and grains per spike were calculated regarding the grain yield of each quadrat.

At Marcos Juarez experiment, on each 0.5 m² quadrat wheat and ryegrass biomass was evaluated four times during the crop cycle (tillering, Zadoks 15–23; stem elongation, Zadoks 3.3;, flowering, Zadoks 6.0; and maturity Zadoks 9). Above ground biomass was harvested by hand and dry weight was determined after drying plants at 65 °C for 48 h. At crop maturity wheat and ryegrass plants were processed such as in the other experiments to determine crop yield, components and ryegrass biomass, spikes and fecundity.

2.3. Measurement of competition

Aggressivity (Snaydon and Satorre, 1989) which takes into account the effect on competition of both crop and weeds was used for measuring competitive ability between species and was calculated as:

Aggressivity = (Bcw/Bc) - (Bwc/Bw)

where Bcw and Bwc are the biomass per unit area at maturity of the crop and weed, respectively, when grown in mixtures and Bc and Bw are their biomass in monoculture (Puricelli et al., 2003).

Relative Yield Total (RYT) shows the intensity of competition and was calculated as:

RYT = Bcw/Bc + Bwc/Bw

RYT value of 1 means full competition between species, and a RYT value of 2 means no competition. Any value of RYT between 1 and 2 means that competition is partial, i.e., that there is some resource complementarity between species (Snaydon and Satorre, 1989; Satorre and Snaydon, 1992).

2.4. Statistical analyses

Data were analyzed by ANOVA and means were compared using LSD test (P < 0.05) corrected by Fisher. Previously Shapiro Wilks and Levene tests were carried out to test the assumptions of ANOVA.

Regression analyses were performed to determine the significance of the relationship between weed density and biomass and the relationship between crop yield, components and ryegrass abundance.



Fig. 1. Ryegrass emergence (%) related to days after crop sowing on the average of all experimental treatments.

3. Results

3.1. Italian ryegrass population and crop emergence

On the average of all experimental conditions, eighty percent of the whole population emerged during the first forty days from crop sowing to 14–21 Zadoks wheat growth stage regardless of N application and experimental site (P > 0.05). In addition, all plants had emerged by eighty days after sowing (Fig. 1). Mean ryegrass emerged seedlings across all trials was 368 (±34) plants m⁻² and there were no differences between fertilized and non-fertilized plots (P > 0.05).

Crop density was 274 ± 11.3 plants m⁻² on the average of the experiments and there were no differences between treatments and experimental areas (*P* > 0.05).

3.2. Italian ryegrass growth

As expected, ryegrass biomass increased with the weed population size. Linear models for biomass related to ryegrass density were significant (P < 0.05) for all the treatments. N application affected ryegrass biomass (P < 0.05) related to population density. When averaged across all the experiments, mean biomass was 1.48 (± 0.05) g and 1.08 (± 0.02) g on fertilized and non-fertilized plots, respectively (Fig. 2). In addition, N increased ryegrass seed production (Fig. 3). Mean fecundity was 190 (± 21) and 118 (± 10.5) (P < 0.05) seeds per plant on fertilized and non-fertilized plots, respectively. However, the number of ryegrass spikes per square meter was not affected by N application. Thus, the number of seeds



Fig. 2. Ryegrass Biomass $(g m^{-2})$ at crop maturity related to ryegrass density (plants m^{-2}) with (\blacksquare) and without N application (Δ) on the average of the experiments.



Fig. 3. Ryegrass seed production (Seeds m^{-2}) related to ryegrass biomass (g m^{-2}) for fertilized (\blacksquare) and no fertilized (Δ) treatments on the average of the experiments.

per ryegrass spike was higher when N was applied than without N fertilization. Ryegrass individuals belonging to the first cohort produced 22 and 28 spikelets per spike (P < 0.05) for non-fertilized and fertilized plots, respectively.

3.3. Competitive effects

When averaged across all the experiments, yield losses with ryegrass densities of 100 plants m⁻² were around 20% and 30% for non-fertilized and fertilized plots, respectively (Fig. 4). Wheat yield was significantly (P < 0.05) related to wheat spikes per square meter (Fig. 5). On the other hand, there was no significant (P > 0.05)relationship between crop yield and grain weight ($r^2 = 0.13$). Averaged over all ryegrass densities, one thousand grain weight was 38.3 (±2.1) g, 31.2 (±1.8) g and 29.8 (±2.1) g. in Marcos Juarez, Azul and Buenos Aires, respectively. In Marcos Juarez there were significant (P < 0.05) differences on competitiveness between cultivars. The cultivar Baguette 13 showed a higher biomass production than Buck Gaucho without ryegrass competition but there was no difference between cultivars in the presence of ryegrass. Across ryegrass densities, crop biomass production at maturity was 24% and 15% lower for Baguette 13 and Buck Gaucho, respectively, compared to biomass production without ryegrass competition (Fig. 6).

3.4. Competitive ability

In all the experimental conditions wheat was more competitive than ryegrass (i.e. aggressivity was higher than 0). Wheat competitiveness decreased when ryegrass density increased and N was applied. Consequently, wheat aggressivity was greater in unfertilized treatments than in fertilized plots. RYT values were not significantly (P > 0.05) different between treatments and were not



Fig. 4. Relative yield of wheat related to ryegrass density in fertilized (\blacksquare) and no fertilized (\Box) treatments on the average of the experiments.



Fig. 5. Wheat yield $(g\,m^{-2})$ related to Spikes m^{-2} on the average of treatments and experiments.

different of 1 (Table 1), showing that there was full competition in all the experimental situations.

4. Discussion

Nitrogen fertilizer has been documented in breaking dormancy of certain weed species (Agenbag and De Villiers, 1989). Egley and Duke (1985) registered that chilling or light requirement for seed germination in some species can be overcome by N, particularly nitrate. Regarding the results presented, N application did not modify the rate and the extension of emergence time (Fig. 1). N was applied forty days after sowing when 80% of the ryegrass had emerged. However, there was no difference in the total amount of seedlings emerged between N conditions. This means N did not increase ryegrass germination. If N had influenced on dormancy, there would be more seedlings and probably more extension of the emergence time. Thus, the results suggest that under field conditions such as those in which the experiments were carried out in this study, weed emergence rate was more related to robust environmental control factors, i.e., temperature and water, than to soil nutrient availability conditions. Similar result was published by Scursoni and Benech Arnold (2002) where no differences were found in wild oat emergence dynamics with different N application regimes (No application, N applied at sowing, N applied at tillering). In addition, Cairns and De Villiers (1986) showed that the optimum dosage rate of ammonia treatment for germination differs between wild oat ecotypes probably due to differences in dormancy status of the seeds. The same rate of ammonia gas was not equally effective in breaking dormancy of semi and deeply dormant wild oat seeds.

The prolonged period of emergence of grass weeds such as ryegrass and wild oat makes it difficult to control the weeds successfully as grass weed herbicides should be applied at a specific weed growth stage. Knowledge of the emergence dynamics of weed populations and competition is the key to achieve both effective weed control and high crop yields. Regarding the results of



Fig. 6. Biomass $(g m^{-2})$ at stem elongation and maturity for wheat Cv. Baguette 13 without competition (black bar), cv. Buck Gaucho without competition (white bar) and the average of both cultivars with ryegrass competition (gray bar). Insert bars are LSD (P < 0.05).

Table 1

Relative Yield of ryegrass and wheat, Aggressivity of wheat and Relative Yield Total (RYT) on the average of Azul and Buenos Aires experiments.

	Ryegrass RY	Wheat RY	Aggressivity	RYT
Ryegrass density (-N)				
100	0.28c	0.85a	0.57a	1.13
200	0.34bc	0.8ab	0.46b	1.14
300	0.3c	0.8ab	0.50ab	1.1
Ryegrass density (+N)				
100	0.45a	0.70bc	0.25c	1.15
200	0.38b	0.62c	0.24c	1
300	0.4ab	0.6c	0.20c	1
LSD ($P < 0.05$)	0.06	0.12	0.10	NS

the present study, the emergence dynamic of ryegrass showed that it would be convenient to control the weed at the beginning of crop tillering because most individuals have already emerged at that time. In addition, the efficacy of some herbicides such as iodosulfurón plus metsulfuron methyl is strongly dependent on the weed growth stage at time of application (Scursoni et al., 2008). Thus, it is not recommendable to delay the application particularly with this sort of herbicides.

N application increased ryegrass competitiveness. In the same way, Ross and Van Acker (2005) reported that nitrogen application increased the competitive effect of wild oat on spring wheat while Dhima and Eleftherohorinos (2001) demonstrated that grain yield reductions of wheat and triticale due to sterile oat interference was higher where 150 kg N ha was applied, compared to the control without N application. The increase in weed competition at higher N has been suggested to be related to an increase in the efficiency of nutrient accumulation and use by weeds (Di Tomaso, 1995). This is in agreement with our results because N fertilization did not modify the germinability of ryegrass and thus, there were not more seedlings in fertilized plots than in non-fertilized ones. There is some evidence that the effect of N fertilization on weed competitiveness is influenced by the growth stage of both crop and weed at time of fertilization. Forcella (1984) documented that rigid ryegrass (Lolium rigidum Gaudin) was less competitive when N was applied before the three-leaf stage of wheat compared with later applications. When N was applied before three leaf stage, wheat used N more rapidly than the weed, tillering sooner and producing larger amount of biomass. Similarly Scursoni and Benech Arnold (2002) showed that wild oat growth was greater when N was applied at crop tillering than at sowing. This suggests the advance of fertilization as a good strategy to achieve less weed growth and increase crop competitiveness resulting not only in lower yield losses but also in lower wild oat fecundity (Scursoni and Benech Arnold, 2002). Nevertheless, there is evidence that the effect of fertilizer rates on weed competitiveness is also species and nutrient dependent. In this sense, the greater surface area of ryegrass roots likely enhanced the competitiveness of the weed relative to wheat under P-deficit conditions (Cralle et al., 2003). In a controlled environment study, shoot and root growth of many agricultural weeds was found to be more responsive to N than that of wheat or canola (Brassica napus L.) Thus, management strategies deserve greater attention when weed infestations consist of species known to be highly responsive to higher soil N levels, such as wild mustard, common lambsquarter or wild oat (Blackshaw et al., 2003).

N fertilization increased individual growth (Fig. 2) and seed production of ryegrass (Fig. 3) suggesting that it is necessary to regard the control of the weed not only because of yield losses but also to prevent more seeds entering the soil seed bank when N is applied. Ryegrass densities of 100 plants m^{-2} reduced wheat yield by 20% and 30% in unfertilized and fertilized plots, respectively

(Fig. 4). This result is in agreement with those published by Monjardino et al. (2003) and Trusler et al. (2007) who suggest wheat yield losses of 22-28% when ryegrass density was 93 plants m^{-2} . As expected, wheat yield was significantly (P < 0.05) related to grain number per square meter which is also significantly (P < 0.05) related to the number of spikes per square meter (Fig. 5). Interestingly, there was no effect of N fertilization either on grain weight or on grains per spike. Across ryegrass densities, thousand grain weight was 33 (± 2.1) g and there were 32 (± 2.2) grains per spike. In Marcos Juarez, there were differences in competitive ability of wheat cultivars. In competition with ryegrass, in terms of the relative yield losses, Buck Gaucho was more competitive than Baguette 13 (Fig. 6). There are not enough recent studies to compare competitive ability of new wheat cultivars in Argentina. However, studies carried out by Satorre and Guglielmini (1990) showed a significant relation between aggressivity and height of the wheat. Similar results were published by Watson et al. (2006) regarding barley cultivars. Full height cultivars may have greater competition for light and higher rate of development than semidwarf cultivars, which implies a more rapid capture of resources.

In relation to competitive indices, as was expected, there was a trend to increase competition when weed density increased, both with and without N application. In addition, the aggressivity of wheat was lower with N fertilization than without it. The RYT indicates there was full competition in all treatments (Table 1). This means that the reduction in the productivity of a component (i.e. crop) is proportional to the capture of resources by the other component (i.e. weed).

These results are of practical concern for farmers in Argentina, because ryegrass is a frequent weed in wheat and barley crops and nitrogen application is an agronomic practice usually used by farmers. Ryegrass was more competitive when N was applied than without N application. This means a reduction in the threshold weed density and thus, it is necessary to anticipate the herbicide application. It would also be favorable to increase crop density in order to reduce ryegrass growth and fecundity. In Argentina, farmers frequently apply N fertilization at tillering but these results show it would be more convenient to apply it earlier during crop cycle in order to increase wheat competitiveness. Manipulation of crop fertilization, crop density and other agronomic practices, such as sowing date, are useful to integrate a weed management strategy that results not only in lower weed population growth but also in lower amount of herbicide application.

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