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#### **Article**

LANGARO, A.C.1\* AGOSTINETTO, D.1 OLIVEIRA, C.1 FRANCO, J.J.<sup>1</sup> ZANDONÁ, R.R.<sup>1</sup> VARGAS, L.2

\* Corresponding author: <langaro.ac@gmail.com>

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## Influence of Nitrogen Fertilization on Herbicide SELECTIVITY IN RICE

Influência da Adubação Nitrogenada na Seletividade de Herbicidas ao Arroz

ABSTRACT - Chemical weed control and nitrogen fertilization are common practices in irrigated rice crops. Nitrogen is an essential element for growth and development of plants and may influence herbicide selectivity. In this study, herbicide selectivity was evaluated according to nitrogen application time in irrigated rice crops. The experiment was conducted in the field in a split-plot design with four replications. Treatments were arranged in a factorial scheme: factor A consisted of different nitrogen application times (without nitrogen, all nitrogen applied before flooding, half of the nitrogen rate applied before flooding and the other half after flooding, all nitrogen applied after flooding); factor B consisted of the application of the herbicides quinclorac, bentazon, bispyribac-sodium and carfentrazone-ethyl. Nitrogen application increased plant height and dry matter accumulation in rice plants, while nitrogen application before flooding and split N applications were favorable to the crop. The herbicide bispyribac-sodium causes the greatest injury and the greatest reduction in rice plant height, followed by bentazon and carfentrazone-ethyl, while the lowest injury and the highest grain yield were caused by the application of quinclorac.

**Keywords:** *Oryza sativa*, nitrogen, selectivity, weeds.

RESUMO - O controle químico de plantas daninhas e a adubação nitrogenada são práticas comuns na cultura do arroz irrigado. O nitrogênio é elemento essencial no crescimento e desenvolvimento das plantas, podendo influenciar na seletividade dos herbicidas. Neste estudo, avaliou-se a seletividade de herbicidas em função da época de aplicação de nitrogênio na cultura do arroz irrigado. O experimento foi conduzido em campo, em delineamento em parcelas subdivididas com quatro repetições. Os tratamentos foram arranjados em esquema fatorial, sendo o fator A constituído por diferentes épocas de aplicação de nitrogênio (sem nitrogênio; todo o nitrogênio aplicado antes da entrada de água; metade do nitrogênio aplicada antes da entrada de água e outra metade depois; e todo o nitrogênio aplicado depois da entrada da água) e o fator B, pela aplicação dos herbicidas quinclorac, bentazon, bispyribac-sodium e carfentrazone-ethyl. A aplicação de nitrogênio resulta em maior estatura e acúmulo de matéria seca nas plantas de arroz, e a aplicação desse nutriente antes da entrada da água e o parcelamento dele favorecem a cultura. O herbicida bispyribac-sodium resulta em maior fitotoxicidade e redução da estatura das plantas de arroz, seguido por bentazon e carfentrazone-ethyl, enquanto a menor fitotoxicidade e a maior produtividade de grãos são decorrentes da aplicação de quinclorac.

**Palavras-chave:** Oryza sativa, nitrogênio, seletividade, plantas daninhas.

<sup>1</sup> Universidade Federal de Pelotas, Pelotas-RS, Brasil. <sup>2</sup> Embrapa Trigo, Passo Fundo, Rio Grande do Sul, Brasil.













#### INTRODUCTION

Rice is considered to be the most economically important product in many developing countries (Duan et al., 2007). With the growing increase in consumption, influenced by population growth, the productive sector is supposed to seek new techniques that can increase production (FAO, 2015).

Among problems commonly found in crop management, weed interference may cause an 80-90% yield reduction of irrigated rice grains (Andres and Machado, 2004). However, these losses can range from 4 to 30% according to genotype, flooding times, plant arrangement used in the crop and herbicide doses in use (Galon et al., 2007; Pinto et al., 2008). In order to avoid losses resulting from competition against weeds, several phytosanitary treatments are used, and chemical control is the main tool to control weeds in irrigated rice fields (Concenço et al., 2006). Another practice that interferes with crop-weed interactions is nitrogen fertilization because it is crucial to plant growth. In addition, it may accelerate plant recovery under the toxic effects of herbicides (Mariot et al., 2003).

Nitrogen (N) is an essential nutrient for all plant species, as it is a key component of several organic compounds, such as chlorophylls, cytochromes, enzymes and coenzymes (Campestrini et al., 2014), which participate in photosynthesis (Taylaran et al., 2011) as well as in dry matter production and accumulation (Feng et al., 2009) and rice panicle formation (Wang et al., 2012; Zhang et al., 2013). It is known that N fertilization is one of the best ways to ensure rice crop productivity (Wang et al., 2012; Zhang et al., 2013). N helps increase the leaf area of rice plants; as a result, intercepted solar radiation and photosynthetic rate become more efficient, thus improving grain yield (Fageria et al., 2003). However, after application, this nutrient can suffer several transformations, and some of them result in losses, mainly by denitrification and volatilization processes (Vahl and Souza, 2004).

N application times are predetermined in two dressings, with half the dose preceding or at the beginning of tillering, at stage  $V_3/V_4$ . The other half should be applied during panicle differentiation (R0 stage); that way, it will provide optimal N supply of in the critical stage (SOSBAI, 2012). The combination of appropriate doses and times not only reduces production costs and environmental pollution, but also results in better performance of irrigated rice, which increases grain yield (Fageria et al., 2011). Thus, the management of nitrogen fertilization, according to application times, can alter the photosynthetic performance of plants during grain filling, thereby affecting the duration and rate of dry matter accumulation (Camargo et al., 2011).

Fertilization timing may influence the behavior of rice plants, because one of the selectivity strategies of herbicides is their lower translocation, as compared to that of weeds (Oliveira Jr. et al., 2011). However, N supply before herbicide application may lead to increased translocation and crop injuries. Therefore, the objective of this study was to evaluate herbicide selectivity according to nitrogen application time in irrigated rice crops.

### **MATERIAL AND METHODS**

The experiment was conducted on the field, using a split-plot design in with four replications; each experimental unit consisted of a subplot with usable area of  $6.12 \text{ m}^2$  (4 x 1.53 m).

The area was prepared according to the conventional rice cultivation system; sowing was carried out on October 31<sup>st</sup>, 2013, using the Puitá INTA CL cultivar at a density of 120 kg ha<sup>-1</sup>. Fertilization was performed by using 300 kg ha<sup>-1</sup> of fertilizer with a 2-20-20 formulation. The plots consisted of nitrogen application times (without nitrogen, all nitrogen applied before flooding, half of the nitrogen applied before flooding and another half after flooding, and all nitrogen applied after flooding); the amount of nitrogen was 120 kg ha, which was calculated according to the soil analysis. Flood irrigation was performed at 22 days after crop emergence (DAE), when plants were at the V4 stage, at two days after herbicide application and one day after N application. The application of 100% nitrogen after flooding was performed three days after the beginning of flood irrigation. The subplots were composed of the herbicides quinclorac (375 g a.i. ha<sup>-1</sup>), bentazon (960 g a.i. ha<sup>-1</sup>), bispyribac-sodium (50 g a.i. ha<sup>-1</sup>) and carfentrazone-ethyl (200 g a.i. ha<sup>-1</sup>), in addition to a control treatment with application of cyhalofop-butyl (315 g a.i. ha<sup>-1</sup>). The herbicides



were applied at 20 DAE; the main weeds in the area were barnyard grass (*Echinochloa* spp.) and cyperaceae (*Cyperus iria* and *Cyperus esculentus*). During panicle differentiation, an additional nitrogen application of 30 kg per hectare was performed throughout the experiment, also with the treatments without N.

Spraying was performed early in the morning, using a  $\mathrm{CO_2}$ -powered continuous-pressure sprayer and a bar with four Teejet 110.015 fan-type nozzles, spaced at 0.5 m, with spraying volume of 120 L ha<sup>-1</sup>. At the time of application, the meteorological conditions were adequate, with relative humidity of 72% and temperature of 21 °C. In order to maintain a weed-free area, cyhalofop-butyl was applied on the total area two days after the herbicides had been applied because a great population of barnyard grass was found.

The evaluated field variables were plant height, shoot dry matter and injury, at 10, 20 and 30 days after the application of the treatments (DAT). At 30 DAT, the number of tillers per plant was also evaluated by manually counting the number of plants and tillers per meter.

In order to determine height, 10 plants per experimental unit were measured. For assessment of injury, visual scores were assigned on a percentage scale, where 0% means no effect and 100% represents the complete death of plants (Frans et al., 1986).

Before the harvest, 10 panicles were collected from the usable area of each experimental unit, in order to determine the number of full and empty grains per panicle. Each panicle was threshed manually, and the full and sterile grains per panicle were counted. Grain yield of rice was determined by harvesting the usable area of plots when moisture content was approximately 22%. Afterwards, the grains were weighed and humidity was determined, data were corrected to 13% moisture. Productivity was expressed in kg ha<sup>-1</sup>. Thousand grain weight was quantified by manually counting eight samples containing 100 grains; the value was corrected to one thousand grains and expressed as grams.

Data were analyzed for homoscedasticity and were subsequently submitted to analysis of variance (p $\leq$ 0.05). In case of statistical significance, the comparison of means was performed by Duncan's test (p $\leq$ 0.05).

#### RESULTS AND DISCUSSION

There was an interaction between herbicides and nitrogen application time for the height and injury variables at 10 DAT (Tables 1 and 4). There was a simple effect of herbicides on height (20 DAT), injury (20 and 30 DAT) and grain yield (Tables 2, 5 and 8). For the variables height (20 and 30 DAT), shoot dry matter (10, 20 and 30 DAT), number of tillers per plant, number of empty grains, thousand grain weight and grain yield, there was a simple effect of nitrogen application time (Tables 3, 6, 7 and 9). For the variable number of grains per panicle, there was no statistical significance (data not shown).

When the effects of the herbicides were compared, the greatest reduction in rice plant height was usually found when bispyribac-sodium was applied, and this effect was found mainly when all nitrogen was applied before flooding (Table 1). At the same time, the application of bentazon and carfentrazone-ethyl also reduced rice plant height.

Bispyribac-sodium is an inhibitor of the acetolactate synthase enzyme, resulting in a reduction in the branched chain amino acid synthesis, such as valine, leucine and isoleucine (Roman, 2007). The phytotoxic effect characterized by chlorosis is typical of herbicides with a physiological action, that is, products with good translocation and that interfere directly in the metabolism of plants, as is the case of bispyribac-sodium (Rodrigues and Almeida, 2011), often resulting in a reduction of plant height.

When nitrogen application times were compared, the greatest height reduction was found for treatments without nitrogen or when all nitrogen was applied after flooding. The smallest plant height in treatments where 100% N was applied after flooding may be due to nutrient losses. When urea is directly applied to water, it is more susceptible to losses caused by ammonium volatilization and denitrification (Humphreys et al., 1987). As a result, a small amount of urea is available to plants, which reduces their height.



 Table 1 - Height (cm) at 10 days after application of herbicides (DAT) to rice plants which received the application of post 

 emergence herbicides and according to nitrogen application time

Height (cm)					
Herbicide		Nitrogen application time			
Heroicide	Without N   Refore water		Half before/ Half after	After water	
Nontreated	28.2 B a	35.7 A a	35.5 A a	27.0 B b	
Quinclorac	30.7 B a	37.7 A a	35.5 A a	30.7 B a	
Bentazon	29.3 BC a	32.9 AB b	35.6 A a	28.0 C ab	
Bispyribac-sodium	25.8 B a	29.9 A c	31.3 A b	25.8 B b	
Carfentrazone-ethyl	27.9 B a	32.6 A b	32.6 A ab	28.8 B ab	

Means followed by lowercase letters in the column differ the herbicides and those followed by capital letters on the line differ the nitrogen application time by Duncan's test ( $p \le 0.05$ ).

At 20 DAT, the rice plants had the lowest height when the plants received the application of bispyribac-sodium and carfentrazone-ethyl; however, they did not differ from the nontreated plants (Table 2). This reduction was also caused by the physiological effect of the herbicide, i.e., growth reduction, as aforementioned. Similar results were found when bispyribac-sodium was applied on upland rice crops at 7 and 14 DAT, causing height reduction (Petter et al., 2011).

When the effect of N application time was evaluated, there was a reduction in plant height in the nitrogen-free treatment at 20 and 30 DAT (Table 3). Among treatments with N, during both evaluation times, there was height reduction when all nitrogen was applied after flooding. Thus, it is evident that, considering the variable plant height, the best nitrogen application time is before flooding, possibly because losses by volatilization and denitrification were smaller.

Table 2 - Height (cm) at 20 days after the application of treatments (DAT) to rice plants which received the application of post-emergent herbicides

Herbicide	Height (cm)
Nontreated	41.8 ab
Quinclorac	45.4 a
Bentazon	43.3 ab
Bispyribac-sodium	40.4 b
Carfentrazone-ethyl	40.8 b
CV (%)	12.13

Means followed by different letters in the column differ by Duncan's test (p $\leq$ 0.05).

**Table 3** - Height at 20 and 30 days after the application of treatments (DAT) to rice plants according to nitrogen application time

Nitua and andication time	Height (cm)		
Nitrogen application time	20 DAT	30 DAT	
Without N	37.1 с	42.9 d	
Before water	45.8 a	58.7 a	
Half before/Half after	44.6 a	53.2 b	
After water	41.9 b	47.3 с	
CV (%)	9.96	9.96	

Means followed by distinct letters in the column differ by Duncan's test ( $p \le 0.05$ ).

As for the variable injury, it was found that all herbicide treatments presented phytotoxic effects, when compared to the nontreated plants (Table 4). Among the herbicides, the greatest injury was found when bispyribac-sodium was applied at all nitrogen application times, but it did not differ from carfentrazone-ethyl when N was fractionated or applied after flooding. When comparing N application times, there was lower bentazon injury when 100% N was applied before flooding or when 50% was applied before and 50% was applied after flooding. The application of bispyribac-sodium caused the highest injury when all nitrogen was applied before flooding. In comparison to the other herbicides, nitrogen application time caused no difference in symptoms.

Thus, it can be inferred that N application before flooding favors the recovery from injury caused by bentazon to rice plants. When the application of bispyribac-sodium was evaluated, there was an inverse behavior, that is, nitrogen application before flooding resulted in greater injury. A possible explanation for this result is the fact that bispyribac-sodium is a systemic herbicide, and its translocation can be increased according to nitrogen absorption. However, it cannot be stated that this was the mechanism which caused greater injuries.



**Table 4** - Injury (%) at 10 days after the application of treatments (DAT) to rice plants which received the application of postemergent herbicides and according to nitrogen application time

	Injury (%)				
Herbicide		Nitrogen application time			
Herbicide	Without N	out N Before water Half be		After water	
Nontreated	0 A c	0 A d	0 A d	0 A d	
Quinclorac	6.3 A b	4.5 A c	4.3 A bc	4.3 A c	
Bentazon	5.0 AB b	3.5 B c	3.5 B c	5.8 A b	
Bispyribac-sodium	8.5 B a	12.5 A a	8.3 B a	7.3 B a	
Carfentrazone-ethyl	6.0 A b	7.5 A b	6.5 A ab	7.3 A a	

Means followed by lowercase letters in the column differ for herbicides and those followed by capital letters in the row differ for nitrogen application times by Duncan's test ( $p \le 0.05$ ).

At 20 DAT, there was higher injury in the crop, resulting from the application of bispyribac-sodium, followed by quinclorac and carfentrazone-ethyl (Table 5). The lowest injury was found with bentazon, which did not differ from the nontreated plants control treatment and was more selective to rice. After evaluation of the effect of herbicides at 30 DAT, it was found that only bispyribac-sodium resulted in injury to the crop. This finding highlights the recovery of plants that were treated with other herbicides. However, the evolution of injury symptoms until the apparent full recovery of plants can still result in reductions in the final yield of the crop because of non-perceptible metabolic disturbances.

In all evaluation times, the highest dry matter increase was found when N was applied before flooding, except at 10 DAT, when there was no difference for split applications of this nutrient (Table 6). The losses resulting from the application of urea in water determined less efficiency with this procedure. These results are in agreement with those found by Humphreys et al. (1987), who also found greater efficiency with the application of urea before the flood, resulting in greater dry matter accumulation.

Average efficiency of N utilization is relatively low in irrigated rice fields, around 40% (Xu et al., 2012). As a result of high consumption of nitrogen fertilizers with low efficiency, a substantial amount of fertilization is lost, causing a series of environmental problems (Xuejun and Fusuo, 2011). N excess is rapidly lost as a result of ammonia volatilization, denitrification, surface runoff and leaching (Wang et al., 2012). Based on the results of dry matter accumulation according to nitrogen application time, it was found that when 100% of nitrogen fertilization is applied before flooding, losses are smaller.

Regardless of nitrogen application time, it was found that there is always a gain in dry matter accumulation, compared to the control treatment without N, and there was a 227% increase in dry matter accumulation when 100% N was applied before flooding, 135% when N

**Table 5** - Injury at 20 and 30 days after the application of treatments (DAT) to rice plants which received the application of post-emergent herbicides

TT 11.11	Injury (%)		
Herbicide	20 DAT	30 DAT	
Nontreated	0 d	0 b	
Quinclorac	2.6 b	0.2 b	
Bentazon	0.9 cd	0.2 b	
Bispyribac-sodium	6.9 a	3.8 a	
Carfentrazone-ethyl	1.7 bc	b	
CV (%)	75.57	118.80	

Means followed by different letters in the column differ by Duncan's test ( $p \le 0.05$ ).

**Table 6** - Shoot dry matter at 10, 20 and 30 days after the application of treatments (DAT) to rice plants according to nitrogen application time

Nitrogen application	Shoot dry matter (g)		
time	10 DAT	20 DAT	30 DAT
Without N	2.34 b	9.29 с	19.30 d
Before water	4.36 a	29.51 a	63.29 a
Half before/Half after	4.01 a	22.25 b	45.45 b
After water	2.34 b	16.79 b	33.70 с
CV (%)	42.72	37.16	30.56

Means followed by different letters in the column differ by Duncan's test ( $p \le 0.05$ ).



was fractionated before and after flooding and 74% when all nitrogen was applied after flooding. A comparison of application times showed that when N is applied before flooding at 30 DAT, dry matter accumulation is 39% higher in comparison to fractioned application, and 87% higher when 100% is applied after flooding.

N application time also resulted in a change in the number of tillers per plant and of empty grains per panicle (Table 7). The lowest tillering was found in the treatment without N and when all nitrogen was applied after flooding, while the largest number of tillers was found in the treatment with 100% N applied before flooding. The application of urea to water increases the ammoniacal N content that is subject to losses, since N is found a few centimeters deep into the soil. As a result, there is less N available, which decreases the number of tillers, N uptake and, consequently, dry matter production (Humphreys et al., 1987).

Table 7 - Number of tillers per plant and empty grains per panicle of rice plants according to nitrogen application time

Nitrogen application time	Plant tiller <sup>-1</sup>	Empty grain per panicle
Without N	6.7 c	7.9 b
Before flooding	11.7 a	8.1 b
Half before/Half after flooding	9.2 b	7.5 b
After flooding	7.2 c	10.6 a
CV (%)	26.59	41.09

Means followed by different letters in the column differ by Duncan's test (p≤0.05).

As for the number of empty grains, it was found that nitrogen applied after flooding resulted in higher sterility (Table 7), possibly because of greater loss of N and lower utilization by plants, since N plays an important role in the formation of the reproductive organs and grains of rice (Barbosa Filho, 1987).

The highest productivity was found when quinclorac was applied. It did not differ from the other herbicides and had better performance than the nontreated plants (Table 8). One of the possible explanations for the fact that the nontreated plants had lower productivity may be the application of one herbicide only, cyhalofop-butyl, which is registered to control monocotyledons. Thus, the presence of broadleaf weeds may have competed against rice, interfering with its grain yield. In addition, cyhalofop-butyl, when applied on dry soil, may cause a reduction in control efficiency.

**Table 8** - Yield of rice plants which received the application of post-emergence herbicides

Herbicide	Yield (kg ha <sup>-1</sup> )
Nontreated	7700.3 b
Quinclorac	9060.7 a
Bentazon	7967.7 ab
Bispyribac-sodium	8315.6 ab
Carfentrazone-ethyl	7901.6 ab
CV (%)	20.64

Means followed by different letters in the column differ by Duncan's test ( $p \le 0.05$ ).

Quinclorac is a hormonal herbicide belonging to the group of synthetic auxins (Roman, 2007). This herbicide is registered to control both barnyard grass and jointvetch (*Aeschynomene* spp.) in irrigated rice crops, thus it has a higher action spectrum in comparison to other herbicides and results in higher grain yield compared to the nontreated plants.

There was a reduction in thousand grain weight and grain yield when nitrogen was not used or when all nitrogen was applied after flooding (Table 9). The application of N on dry or drained soil followed by irrigation has

provided higher grain yield in studies conducted under different climate and soil conditions (Humphreys et al., 1987). The highest productivity in treatments with nitrogen application was already expected, because N increases the leaf area of the plant. Therefore, there is increased efficiency in solar radiation interception and photosynthetic rate and, consequently, in grain yield (Fageria and Stone, 2003).



Table 9 - Thousand grain weight (g) and yield (kg ha<sup>-1</sup>) of rice plants according to nitrogen application time

Nitrogen application time	Thousand grain weight (g)	Yield (kg ha <sup>-1</sup> )
Without N	24.17 b	6802.8 b
Before flooding	25.43 a	9313.7 a
Half before/Half after flooding	25.30 a	9389.2 a
After flooding	24.40 b	7244.7 b
CV (%)	2.84	15.38

Means followed by different letters in the column differ by Duncan's test (p≤0.05).

The production of rice grains is determined by production components, including number of panicles per area, number of grains per panicle and thousand grain weight (Sui et al., 2013), which are directly influenced by fertilizer supply, mainly N (Feng et al., 2009; Zhang et al., 2013). However, variables such as number of panicles and grain weight may not be related to productivity, due to the lack of uniformity in the stand, light and physical space, making it difficult to measure them and consequently providing high coefficients of variation (Oliveira et al., 2002).

Nitrogen application, in general, results in increased height and dry matter in rice plants; nitrogen application before flooding and the split applications (50% before and 50% after flooding) are favorable to the crop. Bispyribac-sodium results in higher injury and reduction of rice plant height, followed by bentazon and carfentrazone-ethyl, while the lowest injury and highest grain yield are due to the application of quinclorac.

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