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Research Article

# Nitrogen availability and glyphosate hormesis on white oat

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**Conflict of Interest:**
The authors declare that there is no conflict of interest regarding the publication of this manuscript.



### Highlights

- Glyphosate commonly induces plant growth at low rates.
- Hormesis is more pronounced at low nitrogen availability.
- Glyphosate hormesis of increases white oat yield by approximately 30%.

### Abstract

**Background:** The effect of low rates of glyphosate has been widely studied in several crops. Low nitrogen stress reduces carbohydrate synthesis and we hypothesize that hormesis from glyphosate occurs at low nitrogen availability.
**Objective:** To evaluate the effects of glyphosate hormesis at different levels of nitrogen on the growth and yield of white oat.
**Methods:** A two-factor factorial (2x7) was conducted in field testing at nitrogen levels (50 and 90 kg $ha^{-1}$) and low rates of glyphosate rates (0 - 180 g a.e. $ha^{-1}$). The glyphosate was applied at the second node already formed. The growth and yield were evaluated.
**Results:** Plant height was not affected by the nitrogen levels. A low rate of glyphosate induced a plant height increase up of around 10%, but the stimulus was no maintained over in the time. Glyphosate hormesis had a greater effect on dry weight under low nitrogen than under high nitrogen conditions. The glyphosate provided a 43% increase in dry weight at a low nitrogen level when applied at rates consistent with a 4.1% field rate. Glyphosate hormesis increased the yield by approximately 30%, and the yield was higher yield under low nitrogen conditions.
**Conclusions:** The plant height stimulus from low doses of glyphosate was not sustained over time. Glyphosate hormesis stimulus persisted and culminated in an increase in dry weight and grain yield. The hormesis effect on dry weight and yield is more pronounced under lower nitrogen availability.

## 1 Introduction

White oats (*Avena sativa* L.) is a multi-purpose cereal integrated as a human food, due to its content of quality protein and soluble fibers. It is also used in animal nutrition, being used for hay, silage, feed composition and forage (Malavolta and Moraes, 2007). This species is an annual grass of the family Poaceae. Relative to the other species of the genus Avena, white oats is demanding in soil fertility and less resistant to drought; however, it is more tolerant to cold, adapting to different regions of the world's temperate climate (Castro et al., 2012). In the 2019 season, Brazil cultivated an area of 396.5 thousand hectares with white oats destined for grain, producing 904 thousand tons of oats, obtaining an average yield of 2280 kg ha$^{-1}$ (Conab, 2019).

Among the factors that interfere in the potential grain yield of oat is the management related of nitrogen fertilization. Nitrogen stimulates tillers and leaf growth, and acts on the structure of proteins in the grains, thus it has an impacting on yield. However, it is necessary to be careful with crops that are grown at high densities, because problems such as plant bedding are frequent (Witkowicz, 2010; Arenhardt et al., 2017). The search for high grain yields with increased nitrogen levels can increase production costs, causing environmental damage due to nitrogen losses, mainly by leaching and volatilization.

Hormesis, is a stimulatory effect on growth of plants under low rates of xenobiotic, and has been noted for a range of herbicides in several plants (Gitti et al., 2011). The low rates of glyphosate may play a key role in plant growth and development, and glyphosate seems to be one of the most studied herbicide in terms of the effects of hormesis in plants. Hormesis is characterized by a biphasic curve, which occurs through the direct stimulatory reaction at low rates of a chemical or through the compensatory response to imbalance in homeostasis. In this way, hormesis can be considered as the homeostatic response to the proposed changes to the plants that acts in the constant maintenance of the metabolism (Cedergreen, 2008).

The effect of low rates of glyphosate on crops has been widely discussed and researched, with the aim of understanding the mechanism by which it acts as a stimulant and its beneficial action on plants (Silva et al., 2009). The hormesis mechanism related to glyphosate has been related to partial inhibition of EPSPs, since glyphosate-resistant soybeans and corn do not exhibit growth stimulus, which is observed in susceptible species (Velini et al., 2008). Another hypothesis is that many herbicides were initially developed as growth regulators, like glyphosate whose predecessor is glyphosine, a compound used as a growth regulator. This herbicide has already shown a hormonal effect in several plants (Souza et al., 2014).

The use of low rates of herbicides has been presented as potentially beneficial to plant growth and able to increase grain yield. Glyphosate is one of the most studied herbicides in terms of its stimulatory effects on plants, and the occurrence of hormesis was found to occur in more than 70% of cases evaluated by Cedergreen et al. (2007). The stimulatory effects of low doses of herbicides are influenced by factors such as the plant species, biotype or cultivar, plant growth stage and physiological status, and plant density or environmental conditions (Belz and Duke, 2014). Most references to glyphosate hormesis refer to changes in morphological characteristics in eucalyptus, soybean, pinus, corn, barley, sugar cane and coffee; nevertheless, effects on crop yield are restricted to a few crops such as barley, beans, *Vicia faba* and *Cicer arietinum* (Silva et al., 2012; Brito et al., 2017).

Thus far, exploration of the use of herbicides to increase crop yield has been restricted to the use of glyphosate as a ripener in sugar cane (Meschede et al., 2010). Research under field conditions pointed to the use of low doses of glyphosate to increased barley yield when applied in grain filling (Cedergreen et al., 2009). However, hormetic effects were more consistent when the plants were grow under suboptimal conditions, such as low nutrient availability of. Therefore, beneficial low doses effects of glyphosate can be used to enhance crops yield without increasing the fertilizer use.

Nitrogen availability of may influence the action of herbicides on weeds and crops, mainly because of itseffect on plant growth and development, which in turn interferes with physiological and biochemical processes such as the absorption, translocation and metabolism of herbicides (Mithila et al., 2008). Dickson et al. (1990) reported that *Avena sativa* is more tolerant to the effects of the herbicides fluazifop and glyphosate when the plants are grown in an environment with low nitrogen availability. Low nitrogen stress reduces carbohydrate synthesis and translocation in plants, which reduces glyphosate translocation and efficacy (Mithila et al., 2008).

Thus, our hypothesis in this study is that low rates of glyphosate stimulate the growth of white oat when nitrogen availability is low. Therefore, the objective was to evaluate the effects of glyphosate hormesis at different levels of nitrogen on the growth and yield of white oat (*Avena sativa* L.).

## 2 Materials and Methods

The experiment was carried out in the field during June and November of 2016, at Universidade Federal de Santa Maria, Frederico Westphalen, RS, Brazil. The soil was classified as typical Aluminoferric Red Latosol (Oxisol), with the following physico-chemical characteristics, Clay = 60%, pH 6.2, SMP = 6.2, P = 4,5 mg dm$^{-3}$, K = 120.5 mg dm$^{-3}$, MO = 3.2%, Al = 0 cmol dm$^{-3}$, Ca = 8.7 cmol dm$^{-3}$, Mg = 4.6 cmol dm$^{-3}$, CTC = 16.6 cmol $dm^{-3}$, H + Al = 3.0 cmol $dm^{-3}$, Base Saturation = 81.9%. During the experiment, the mean temperature were 11.0; 14.6; 16.3; 16.4; 19.3 and 21.1 ºC and the total rainfall was 30.4; 121.4; 163.0; 70.0; 289.6 and 116.0 mm to June, July, August, September, October and November, respectively.

The experimental design was a randomized complete block design with a two-factor factorial treatment arrangement with three replications. The factors were the nitrogen levels and the glyphosate rates. The nitrogen level applied were 50 and 90 kg $ha^{-1}$ of nitrogen (N) and the rates of glyphosate represented 0; 3.6; 7.2; 18; 36; 72 and 180 g a.e. $ha^{-1}$. Nitrogen fertilization levels of 50 and 90 kg $ha^{-1}$ were based on the potential grain yield of 3.0 and 5.0 t $ha^{-1}$, respectively. Each plot was composed of 3.5 meters long by 1.87 meters wide. The experimental area was burndown with glyphosate (720 g a.e. $ha^{-1}$) at 20 days before planting and paraquat (400 g a.i. $ha^{-1}$) at planting day.

The white oat (URS Taura) was established at 330 plants $m^{-2}$, in rows spaced 0.17 m apart and fertilization was 200 kg of NPK 5-25-25. The nitrogen treatments were applied at 40 and 80 kg $ha^{-1}$ of N at $V_3$ stage of white oat, with urea as the source. Post-emergence weed management was performed with metsulfuron-methyl (5 g a.i. $ha^{-1}$) applied in the V4 stage and removed at weekly intervals manual handle. Disease management was carried out with the application of propiconazole (125 g a.e. $ha^{-1}$) applied in the flowering stage.

The glyphosate rates was applied to white oat once the second node had formed, using a $CO_2$-pressurized backpack sprayer equipped with 110015 flat-fan spray nozzles at 100 kPa calibrated to deliver 150 L $ha^{-1}$.

The plant height was evaluated at 23 and 38 days after application (DAA), determined by randomly selecting 10 plants within the plot, each from ground to the tip of the top-most fully expanded leaf. At 38 DAA, plants were collected at 50 cm along the sowing line to evaluate the dry weight of plants. The plants were placed in paper bags and dried in an oven with forced air circulation at 60 ºC for 72 hours and weighed to determine the dry weight. The weight of a thousand grains (WTG) was determined by harvesting 10 random panicles in the plots and counted in six subsamples 100 seeds. Plots were harvested by hand to determine yield in 6 rows by 2.4 m long. The yield were adjusted to 13% moisture and expressed in kg $ha^{-1}$.

The data were submitted to analysis of variance by the F test, with p<0.05 for the main effects and p<0.10 for the factors interactions. Data on factorial interactions were fitted to the non-linear regression component of the Lorentzian four-parameter model (Jasper et al., 2016) (equation 1), which were plotted using SigmaPlot.

$$y = y0 + \frac{a}{1+\left(\frac{x-x0}{b}\right)^2} \qquad \text{(eq. 1)}$$

where y is the response variable; y0 is the response of y when the rate tends to infinity; a is the value corresponding to the increase in y in rate x0; x0 is the value that expresses the upper limit for y; b corresponds to the mean value between upper and lower limit.

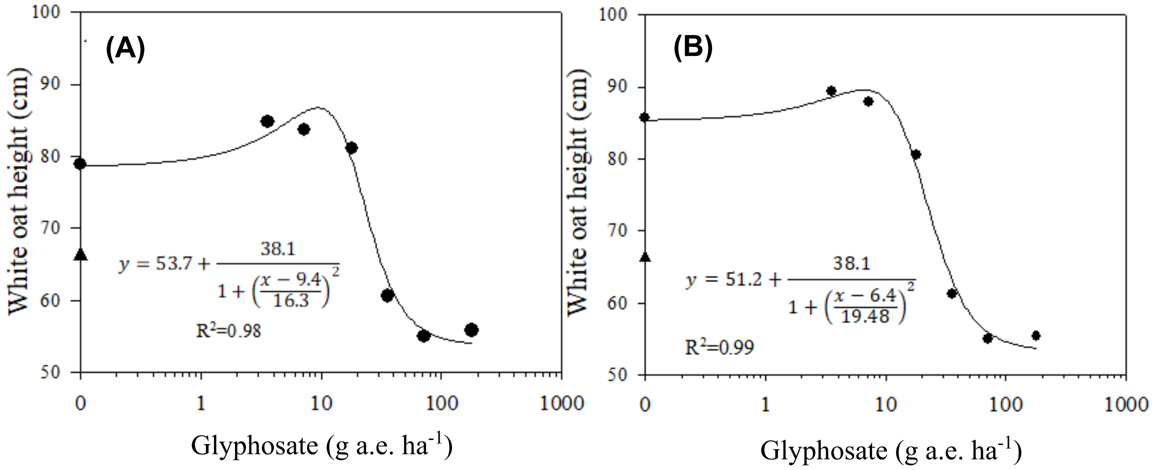
## 3 Results and Discussion

There was an interaction between the nitrogen level (N) and the glyphosate rate with respect to dry weight and yield. For plant height at 23 and 38 DAA, and WTG an effect was only verified for the glyphosate rate (Table 1). The Lorentzian model was a good fit for the white oat data, considering the high coefficients of determination observed (Figures 1 and 2).

Stimulatory effects of low rates of glyphosate on the height of height of white oat were observed, where plant height increased by 18 g a.e. $ha^{-1}$ of glyphosate at 23 AD, while at 38 DAA the increase occurred up to 7.2 g a.e. $ha^{-1}$ (Figure 2). The maximum plant height was estimated for rates of 9.4 and 6.4 g a.e. $ha^{-1}$, at 23 and 38 DAA, respectively. Regarding untreated plants, there were no losses in plant height with glyphosate rates ranging from 0 at 18 g a.e. $ha^{-1}$ at 23 DAA, and 7.2 g a.e. $ha^{-1}$ at 38 DAA. In contrast, when glyphosate

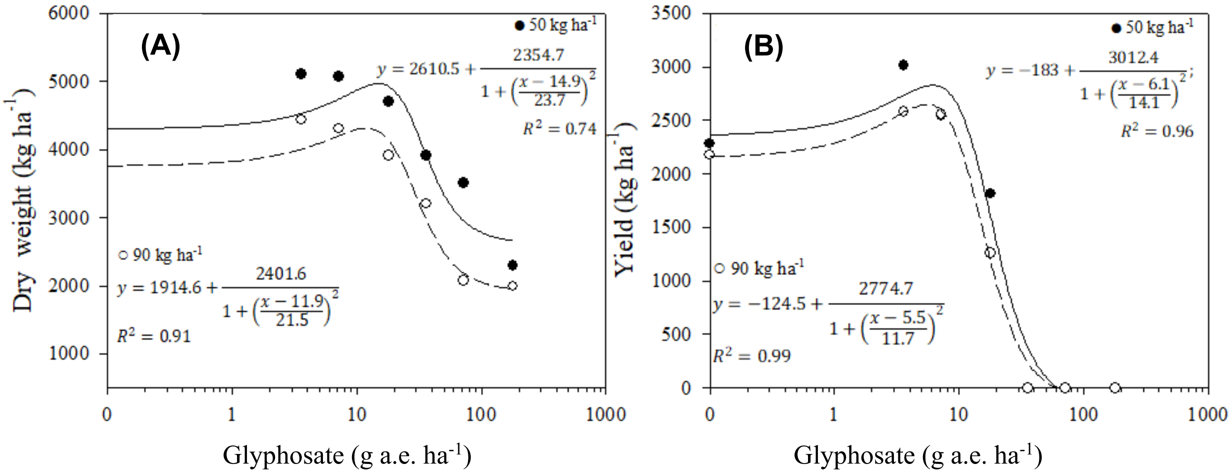
**Table 1** - Analysis of variance and F values

| Source of variance | Plant height (23 DAA) | Plant height (38 DAA) | Dry weight | Yield | Weight of a thousand grains |
|---|---|---|---|---|---|
| Nitrogen (N) | 0.486 | 0.430 | 0.005 | 0.015 | 0.524 |
| Glyphosate rates (GR) | <0.001 | <0.001 | 0.0021 | <0.001 | <0.001 |
| Blocks | 0.146 | 0.501 | 0.183 | 0.022 | 0.154 |
| N*GR | 0.795 | 0.489 | 0.095 | 0.087 | 0.687 |
| CV% | 4.8 | 4.8 | 18.6 | 13.4 | 8.4 |




▲ Indicate white oat height at glyphosate application. $y=y0+(a/1+((x-x0)/b)^2)$, where $y0$ is the response of y when the rate tends to infinity; $a$ is the value corresponding to the increase in y in rate $x0$; $x0$ is the value that expresses the upper limit for $y$; $b$ corresponds to the mean value between upper and lower limit.

**Figure 1** - Plant height of white oat under low rates of glyphosate at 23 (A) and 38 (B) days after application.




$y=y0+a/1+((x-x0)/b)^2$, where $y0$ is the response of y when the rate tends to infinity; $a$ is the value corresponding to the increase in y in rate $x0$; $x0$ is the value that expresses the upper limit for $y$; $b$ corresponds to the mean value between upper and lower limit.

**Figure 2** - Dry weight and yield of white oat under nitrogen levels and low rates of glyphosate.

was applied at up to 36 g a.e. $ha^{-1}$, it inhibited the plant height compared to 0 DAA. The reduction in plant height with low rates of glyphosate may be associated with the effects of inhibition of branched-chain amino acids, especially tryptophan, which is the a precursor of the synthesis of the growth hormone indolelacetic acid (Kruse et al., 2000).

The effect of hormesis on the plant height was not maintained at the time of evaluations were carried out: an increase of 9.7% was verified at 23 DAA and a decline to 4.2% at 38 DAA (Figure 1). This behavior was also reported for *Brachiaria brizantha* under the effects of glyphosate hormesis (Nascentes et al., 2015). In barley, the glyphosate hormesis effect occurs in the first week, but the initial growth increase is followed by a slight decline to levels below those of control plants (Cedergreen, 2008).

The results observed in white oat are similar to those obtained for *Brachiaria brizantha*, which showed stimulus in height between rates of 16 to 21 g a.e. $ha^{-1}$ of glyphosate (Nascentes et al., 2015). In sugarcane, the highest glyphosate hormesis effect on tiller height was at the rate of 1.8 g a.e. $ha^{-1}$, which was an increase of 13% compared with the untreated control, while that rates of 3.6 and 7.2 g a.e. $ha^{-1}$ did not affect tiller height (Silva et al., 2009). Plant height is also influenced in cotton crop, in which there is a stimulus of 16.4% in height at a rate of 27 g a.e. $ha^{-1}$ (Neves, 2009).

White oat showed no difference in dry weight between N levels on untreated plants with glyphosate; however, the low rates of glyphosate increased the accumulation of dry weight in plants grown at lower N levels (Figure 2). A similar response has been reported in barley, where glyphosate hormesis shows greater increases in plants grown under low N vs. high N availability (Cedergreen et al., 2016).

The glyphosate rate of 14.9 g a.e. $ha^{-1}$ provided the highest increase in dry weight under 50 kg $ha^{-1}$ of N, about 43% of the untreated plants (Figure 2). At the level of 90 kg $ha^{-1}$ nitrogen, the maximum accumulation of dry weight occurred at rate of 11.9 g a.e. $ha^{-1}$, corresponding to a 31% increase in relation to the control. The maximum dry weight accumulation was 15% higher when the plants were cultivated at the lowest nitrogen level. In addition to the higher accumulation of dry weight at the lower N level, a smaller decrease in dry weight was also observed at the highest rate of glyphosate, which presented dry weight of 2610.5 and 1914.6 kg $ha^{-1}$, for N levels of 50 and 90 kg $ha^{-1}$, respectively.

These results may be associated with low photoassimilated synthesis and its translocation under conditions of low N availability, which impacts the movement of glyphosate to the meristem and, consequently, the inhibition of EPSPs (Mithila et al., 2008). Thus, low rates of glyphosate are sufficient to decrease EPSPs activity, which results in reduction of the designated carbon displacement to the shikimic acid pathway (Meschede et al., 2010). In addition, under these conditions, the plant increases its metabolic activity, presumably to activate detoxification, inactivation, or compartmentalization processes (Reddy et al., 2008). Other factors are linked to increased carbon fixation efficiency and reduced lignin synthesis. The increase in photosynthesis of glyphosate-treated plants is due to increased Rubisco activity, RuBP regeneration, or triose phosphate (Cedergreen and Olesen, 2010). Lignin formation is synthesized from phenylalanine by various cinnamic acid derivatives, which are produced by the shikimic acid route, and with partial inhibition of EPSPs changes in lignin balance can occur in plants (Taiz and Zeiger, 2013). The reduction in lignin biosynthesis can leadi to enhanced cell wall elasticity, which in turn would result in great longitudinal growth of the plant (Belz and Duke, 2014).

Studies have been conducted on other cereals such as barley, maize, and poaceae family such as *Brachiaria decumbens*, *B. brizantha*, *Echinochloa crus-galli* (Brito et al., 2017). Low rates of glyphosate had the effect of increasing barley dry weight by approximately 33% (Cedergreen, 2008). However, in applications close to the reproductive period, there was no hormonal effect on dry weight (Cedergreen et al., 2009). In maize, the dry weight increment occurred between 1.8 and 36 g a.e. $ha^{-1}$ ofglyphosate, with a 25% increase in shoot dry weight, estimated at 22.6 g a.e. $ha^{-1}$ (Velini et al., 2008). In *B. brizantha* the maximum increase in dry weight was 31% observed at 15 days after application of 10.5 g a.e. $ha^{-1}$ of glyphosate (Nascentes et al., 2015).

Hormesis-related studies mostly evaluate short-term plant development, and do not produce consistent evidence that the stimulatory effect can lead to a significant increase in crop yield (Brito et al., 2017). However, in the present study, the results obtained for the yield of white oat indicate that glyphosate hormesis stimulus persists and culminates in an increase in grain yield (Figure 2). Glyphosate hormesis was higher in white oat at lowest N level, with an estimated maximum yield of 2828.5 kg $ha^{-1}$ at a rate of 6.1 g a.e. $ha^{-1}$, while for the highest level of N, the rate for the highest yield (2650.2 kg $ha^{-1}$) was 5.5 g a.e. $ha^{-1}$. In barley, the application of low rates of glyphosate between 2.5 and 20 g a.e. $ha^{-1}$ at the ear appearance induced a 27-36% increase in the number of $m^{-2}$ grains, which yielded a grain yield 12% higher than the untreated control (Cedergreen et al., 2009). In beans, the rate of 10 g a.e. $ha^{-1}$ of glyphosate provided an increase in yield of up to 10% (Silva et al., 2012).

The WTG did not present a hormesis effect as a function of glyphosate application, however, it was observed that at rates higher than 7.4 g a.e. $ha^{-1}$ there was no grain formation (data not show). These effects are in agreement with the results obtained in corn crop, where glyphosate rates showed no increase in the weight of a thousand grains in comparison with untreated plants (Souza et al., 2014).

Herbicide hormesis can be accompanied by undesirable effects in non-target plants, among which are the induction of pathogen defenses in plants (Dann et al., 1999), the evolution of herbicide resistance (Belz et al., 2018), an increase in the reproductive capacity of weeds (Gomes, 2014), and an increase in competitive weeds (Abbas et al., 2016). The application of low rates of glyphosate has a positive impact on the development and yield of crops; however, their use as a form of agronomic recommendation should be done very carefully, since the results can be variable depending on the rates, crops stages, climatic conditions and nutritional status of plants (Belz and Duke, 2014; Cedergreen et al., 2016).

## 4 Conclusions

Overall, nitrogen fertilization did not influence glyphosate hormesis effects on the height of white oat plants. The low rates of glyphosate slightly stimulated the initial increase in plant height, but the effects was not sustained over time. The dry weight and grain yield were increased by the low rates of glyphosate, being more pronounced with fertilization of 50 kg ha$^{-1}$ of nitrogen. Nitrogen and low rates of glyphosate did not influence the grains weight of white oat.

## 5 Contributions

All authors contributed equally for the experiment conduction, statistical analysis and writing of the manuscript.

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