

Selectivity of 2,4-D salts applied at different phenological stages of wheat cultivars

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ABSTRACT: The selectivity of the herbicide 2,4-D depends on the plant species, the formulations produced and/or the developmental stages of the plants to which it is applied. The objective of this study was to assess the best stage of development of wheat cultivars for the application of 2,4-D salt formulations. The experimental design consisted of randomized blocks arranged in a factorial scheme ($2 \times 2 \times 4 + 1$) with four replicates. The treatments evaluated were wheat cultivars (OR 1403 and TBio Toruk), 2,4-D formulations (amine and choline salts) and crop development stages (before tillering start, at full tillering, first node and booting), another control without herbicides. 2,4-D choline and amine cause greater phytotoxicity when applied at the phenological stage of the first node of the cultivar OR 1403 and less at TBio Toruk. The highest number and length of ears, number of full grains, thousand grain weight, hectoliter weight, grain productivity and lowest number of sterile grains generally occurred when 2,4-D-choline was applied at the first node of the cultivar OR 1403 and when 2,4-D-amine was applied at the rubberization of TBio Toruk. The highest grain yields occurred when 2,4-D-choline and amine were applied to varieties in wheat booting.

Key words: auxinic herbicide; dichlorophenoxyacetic acid; *Triticum aestivum*

Seletividade de sais de 2,4-D aplicados em diferentes estádios fenológicos de cultivares de trigo

RESUMO: A seletividade do herbicida 2,4-D depende das espécies de plantas, das formulações que é produzido e/ou dos estádios de desenvolvimento das culturas em que é aplicado. O objetivo deste estudo foi avaliar o melhor estágio de desenvolvimento de cultivares de trigo para aplicação de formulações de sais de 2,4-D. O delineamento utilizado foi de blocos casualizados, arranjado em esquema fatorial ($2 \times 2 \times 4 + 1$), com quatro repetições. Os tratamentos avaliados foram, cultivares de trigo (OR 1403 e TBIO Toruk), formulações de 2,4-D (sal amina e colina) e estádios de desenvolvimento da cultura (antes do perfilhamento, no perfilhamento, no primeiro nó e emborrachamento), mais uma testemunha sem herbicidas. O 2,4-D colina e amina ocasionam maior fitotoxicidade ao serem aplicados no estágio fenológico de primeiro nó da cultivar OR 1403 e menor na TBIO Toruk. O maior número e comprimento de espigas, número de grãos cheios, peso de mil grãos, peso hectolitro, produtividade de grãos e menor número de grãos estéreis, em geral, ocorreu ao se aplicar o 2,4-D colina no primeiro nó da cultivar OR 1403 e no emborrachamento da TBIO Toruk ao se usar o 2,4-D amina. As maiores produtividades de grãos do trigo ocorreram ao se usar o 2,4-D colina e amina, nas cultivares OR 1403 e TBIO Toruk, no emborrachamento.

Palavras-chave: herbicida auxínico; ácido diclorofenoxiacético; *Triticum aestivum*



Introduction

Weeds compete with crops for environmental resources such as nutrients, water, light, and space (Galon et al., 2021; Kubiak et al., 2022), increasing production costs and reducing the producers profit. When these are not controlled, they cause wheat grain losses of 18 to 82% (Gharde et al., 2018; Galon et al., 2021), as well as being hosts to insects, diseases and releasing allelopathic substances that interfere with the growth and development of the crop (Gharde et al., 2018; Kubiak et al., 2022).

Among the main weed species that infest wheat are turnip and radish (*Raphanus raphanistrum* and *R. sativus*), horseweed (*Conyza bonariensis*, *C. canadensis*, and *C. sumatrensis*), common sowthistle (*Sonchus oleraceus*), purple loosestrife (*Echium plantagineum*), 'cipó-de-veado' (*Polygnum convolvulus*), ryegrass (*Lolium multiflorum*), and white and black oats (*Avena sativa* and *A. strigosa*) (Costa & Rizzardi, 2015; Bajwa et al., 2016; Roso et al., 2017; Galon et al., 2021).

Turnip/radish sprouts are a dominant feature of winter crops in southern Brazil, mainly because they are used as pasture or even as mulch (after the soya harvest until winter crops are sown). These species are also used to adopt the no-till system, promoting an increase in the soils seed bank (Costa & Rizzardi, 2015). In Brazil, there have already been cases of resistance of turnip and/or radish plants to herbicides that inhibit the enzyme aceto lactate synthase - ALS (Cechin et al., 2016; Heap, 2024), making it more difficult to adopt chemical control in wheat cultivation with this mechanism of action.

The *Conyza* species (*C. bonariensis*, *C. canadensis*, and *C. sumatrensis*) are among the most problematic, harmful, invasive, and widely distributed in agriculture worldwide (Bajwa et al., 2016). In Brazil, horseweed have infested both crops sown in winter and those grown in summer, which requires managing these species all year round (Piasecki et al., 2019). These species have already been described as being resistant to herbicides that inhibit EPSPs (enol pyruvyl shikimate phosphate synthase), ALS, photosystems I and II, synthetic auxins, and Protox (protoporphyrin oxidase) (Heap, 2024). These herbicides are commonly used to desiccate areas for planting wheat, or even in the pre- or post-emergence of the crop, thus limiting their use in weed control and restricting the number of products available as a possible alternative to chemical management.

In view of the many cases of weed biotypes showing resistance to ALS, ACCase and EPSPs inhibitor herbicides, these mechanisms of action being widely used for control during desiccation or after sowing wheat, 2,4-dichlorophenoxyacetic acid, better known as 2,4-D, has currently been used, especially for the control of Eudicotyledons. This herbicide is highly effective, low-cost, selective to grasses and an option for controlling plants that are resistant to other mechanisms of action, such as turnip, radish, purple loosestrife or even horseweed species (Cechin et al., 2016; Roso et al., 2017; Piasecki et al., 2019; Heap, 2024).

2,4-D is known as a growth regulator, synthetic auxin or hormonal herbicide because it is part of the class of auxin mimetics and, at low concentrations, it can promote physiological and biochemical effects similar to those of indole-3-acetic acid (IAA), the main auxin present in plants (Mithila et al., 2011). However, the use of 2,4-D to control weeds can cause damage to cereals such as wheat. These effects are mainly related to the formulation and dose of the product, as well as the stage of development, crop cultivars, and soil and weather conditions at the time of application (Kumar & Singh, 2010; Peterson et al., 2016; Skelton et al., 2017; Soligo et al., 2022).

Among the direct and indirect effects already described by the use of 2,4-D are phytotoxicity, changes in nutrient absorption, deregulation of plant defense mechanisms, and a reduction in grain yield components (Kumar & Singh, 2010; Piasecki et al., 2017; Skelton et al., 2017; Rüdell et al., 2021; Soligo et al., 2022). The herbicide 2,4-D can be marketed in amine, choline, and ester salt formulations, the latter of which is no longer marketed in Brazil (Contiero et al., 2016). Amines are the most widely used in the world due to their greater solubility in water and adsorption to the soil, as well as their lower volatility compared to ester formulations (Roman et al., 2006). In addition, the amine salt requires less time to cross the leaf cuticle than the other salts (Contiero et al., 2016).

The formulation of 2,4-D from choline salt has emerged as a new technology capable of controlling weed species resistant to herbicides, including glyphosate (Sosnoskie et al., 2015). The formulation of 2,4-D choline salt features Colex-D technology and has the differential of reducing the percentage of finer droplets, ultra-low volatility, reduced odor, and ease of handling when compared to other formulations (Sosnoskie et al., 2015; Skelton et al., 2017). The lower volatility of 2,4-D choline, however, can influence its retention on leaves and promote leaf damage depending on the stage of crop development at which it is applied (Oliveira et al., 2019).

Studies of 2,4-D formulations are extremely important in order to gain a better understanding of its efficacy, speed, control spectrum, selectivity, and toxicity in different crops (Kumar & Singh, 2010; Soligo et al., 2022). The selectivity of auxin-mimicking herbicides to wheat and other grasses, including 2,4-D, is due to mechanisms such as low leaf penetration, limited translocation in the phloem, insensitivity at the site of action, and low metabolization of the herbicides (Roman et al., 2006; Peterson et al., 2016). In addition, herbicide tolerance depends on a number of factors such as the formulation of the products and the dose applied, the stage of development and cultivars of the crop, environmental and soil conditions (Roman et al., 2006; Peterson et al., 2016; Soligo et al., 2022).

For weed control in wheat, 2,4-D should be applied until the first visible node appears (Franco & Evangelista, 2018). Very early applications of 2,4-D to wheat, i.e. to newly emerged seedlings, can lead to defective ears, curling of the leaves and a reduction in height. This is because these herbicides attack the apical meristem. As a result, the most typical symptom

of 2,4-D phytotoxicity is the retention of the ears on the stalk after the elongation stage, directly interfering with flower differentiation. The application of hormonal herbicides at a stage close to anthesis can reduce wheat grain yield by up to 60% (Rodrigues et al., 2006).

Given the scarcity of studies comparing the effect of applying different formulations of 2,4-D salts to wheat, the hypothesis of this research is that plants of this cereal may show differential responses in terms of selectivity to cultivars and in grain yield components when exposed to different salts of 2,4-D (amine or choline salt), at the stages of development before tillering, at full tillering, at the first node, and booting. In this sense, the objective of this study was to assess the best stage of crop development for applying formulations of 2,4-D salts (amine and choline) to the wheat cultivars OR 1403 and TBIO Toruk.

Materials and Methods

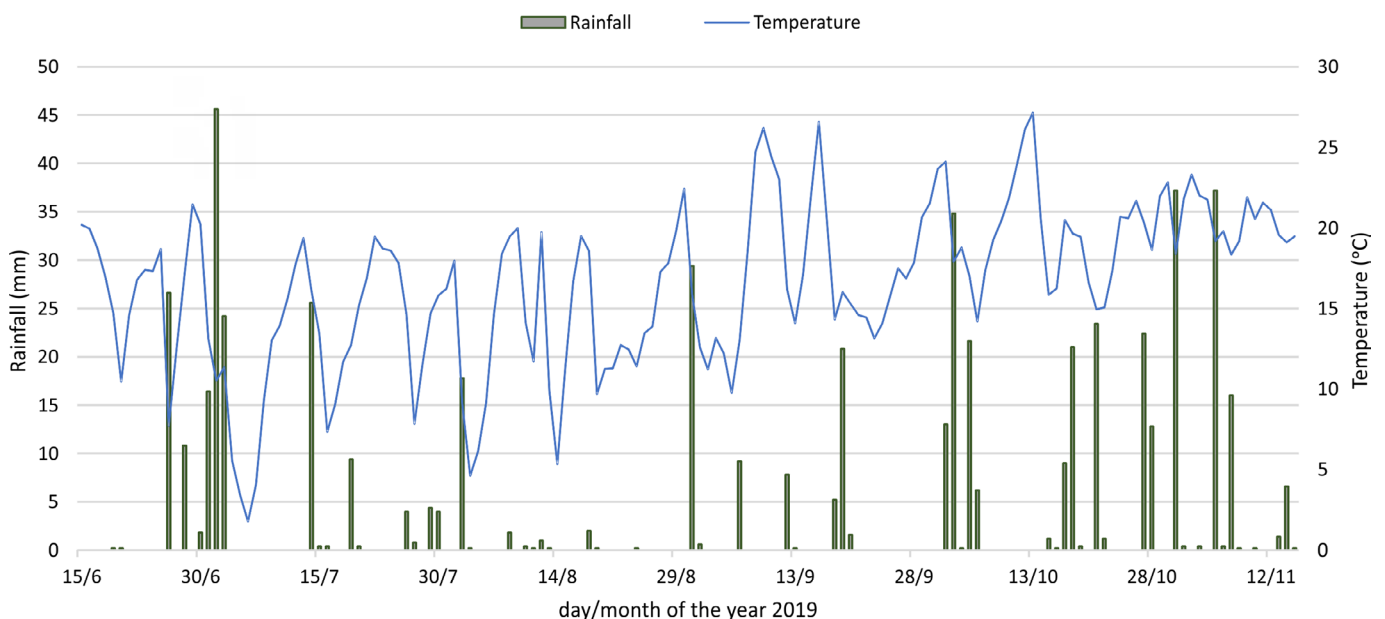
The experiment was set up in the field, in the experimental area of the Universidade Federal da Fronteira Sul (UFFS), Erechim/RS, Brasil, from June to November 2019, at geographical coordinates 27° 43' 26.54" S, 52° 17' 42.42" W and altitude of 775 m. The soil in the area is classified as Latossolo Vermelho Aluminoférrico típico (Santos et al., 2018). The climatic conditions (temperature, humidity, and rainfall) during the experiment period are shown in Figure 1.

The wheat was sown in a direct sowing system and the vegetation was desiccated 15 days before the work was carried out with glyphosate + saflufenacil (1,080 + 70 g ha⁻¹) + mineral oil (0.5% v/v). Soil fertility was corrected based on the chemical analysis and following the fertilization recommendations for

the wheat crop (CQFS-RS/SC, 2016). The physico-chemical characteristics of the area observed in the soil analysis were: pH in water of 4.7; OM = 3.04%; P = 10.05 mg dm⁻³; K = 106 cmol_c dm⁻³; Al³⁺ = 1.0 cmol_c dm⁻³; Ca²⁺ = 5.1 cmol_c dm⁻³; Mg²⁺ = 4.1 cmol_c dm⁻³; CTC_{effective} = 9.9 cmol_c dm⁻³; CTC_{pH7} = 18.60 cmol_c dm⁻³; H + Al = 9.70 cmol_c dm⁻³; base saturation = 48%; and, clay = 64%.

The experiment was sown on 13/07/2019 using the medium-cycle cultivars OR 1403 and TBIO Toruk using a seeder/adubator. The average seed density was 330 m⁻², which resulted in a final population of approximately 3,300,000 plants ha⁻¹. The base fertilizer was 298 kg ha⁻¹ of the 8-25-20 of N-P-K formula. When the wheat cultivars were at the tillering and elongation stage, a total of 48.16 kg ha⁻¹ of nitrogen was applied as top dressing, divided into two applications of 53.51 kg ha⁻¹ of urea.

The experiment was set up in a randomized block design, arranged in a trifactorial scheme (2 × 2 × 4 + 1), with four replications. Factor A was made up of the wheat cultivars (TBIO Toruk and OR 1403), B of the 2,4-D formulations (choline and amine salt), and C of the crop development stages at which the herbicides were applied (before tillering, at full tillering, at the first node, and booting), plus a control without herbicide application. The treatments used in the experiment are described in Table 1. It should be noted that only 2,4-D amine is recommended for application to wheat at doses ranging from 0.40 to 2.00 L ha⁻¹ of commercial product, while 2,4-D choline is not registered for use on this crop (AGROFIT, 2024). However, 2,4-D choline was used in the experiment as a possibility for future application, as it has fewer problems with drift and volatilization among hormonal herbicides (Contiero et al., 2016). The wheat cultivars TBIO Toruk and OR



Source: INMET (2024).

Figure 1. Average temperature (°C), humidity (%), and rainfall (mm) during the period of the experiment, from June to November 2019.

Table 1. Treatments used in the experiment, respective stages, and doses applied to the wheat crop as a result of using formulations of the herbicide 2,4-D on the wheat cultivars OR 1403 and TBIO Toruk. UFFS/Erechim/RS, Brazil.

| Cultivars | Treatments | Type of salt | Commercial product | Dose P.C (L ha ⁻¹) | Dose e.a (g ha ⁻¹) | Wheat stage |
|------------------------|--------------------|-----------------|--------------------|--------------------------------|-------------------------------------|-------------------------------------|
| OR 1403 and TBio Toruk | Without herbicides | --- | --- | --- | --- | Before tillering |
| | Without herbicides | --- | --- | --- | --- | At full tillering |
| | Without herbicides | --- | --- | --- | --- | After emission 1 st node |
| | Without herbicides | --- | --- | --- | --- | Booting |
| | 2,4-D | Amine | Aminol 806 | 1.00 | 670 | Before tillering |
| | 2,4-D | Amine | Aminol 806 | 1.00 | 670 | At full tillering |
| | 2,4-D | Amine | Aminol 806 | 1.00 | 670 | After emission 1 st node |
| | 2,4-D | Amine | Aminol 806 | 1.00 | 670 | Booting |
| | 2,4-D | Choline | Enlist® Colex-D | 1.48 | 450 | Before tillering |
| | 2,4-D | Choline | Enlist® Colex-D | 1.48 | 450 | At full tillering |
| 2,4-D | Choline | Enlist® Colex-D | 1.48 | 450 | After emission 1 st node | |
| 2,4-D | Choline | Enlist® Colex-D | 1.48 | 450 | Booting | |

1403 were chosen for the trial because they were the most widely sown in the region covered by the study and in order to observe differences in selectivity to the herbicides tested.

Each experimental unit had dimensions of 5.00 × 2.72 m, totaling an area of 13.60 m², containing 16 sowing lines spaced 0.17 m apart. The useful area of the plots was 6.80 m² (4.00 × 1.70 m). The ten central rows were harvested, discarding three rows on each side (borders) and 0.50 m at the headlands (beginning and end of the plot) for analysis.

The herbicides were applied using a CO₂ pressurized precision knapsack sprayer with a boom equipped with four DG 110.02 fan spray tips, maintaining a constant pressure of 210 kPa and a travel speed of 3.6 km h⁻¹, which provided a flow rate of 150 L ha⁻¹ of herbicide mixture. The environmental conditions at the time the treatments were applied to the wheat crop can be seen in Table 2.

Assessments of phytotoxicity to wheat cultivars were made visually at 7, 14, 21, 28, and 35 days after treatment (DAT). For this purpose, percentage scores were assigned from 0 to 100%, with a score of zero (0%) corresponding to treatments with no damage to the crop and a score of 100 (100%) to the death of the wheat plants, in accordance with the methodology of the SBCPD (1995).

The variables assessed during the pre-harvest of the wheat crop were the number of ears per area (m²), ear length (cm), and the number of full and sterile grains per ear. The number of ears m⁻² was determined in the center of each experimental unit using a 0.50 × 0.50 m PVC square. The length of the ears was measured with a ruler graduated in centimeters. The number of full and sterile grains in the ears was determined

by counting. Ten wheat plants were randomly harvested from the useful area of each experimental unit to determine the length of the ears and the number of full and sterile grains.

When the wheat was at 15% moisture, it was harvested by hand from a 6.8 m² area and then threshed with a plot thresher. The hectolitre weight, thousand grain weight, and grain yield were then determined. The hectolitre weight - Ph (kg hL⁻¹) was determined using a Dalle Molle model 40 scale. The thousand grain weight (g) was measured by counting eight samples of 100 grains each, for each treatment, using an analytical balance for weighing. Grain yield was then estimated in kg ha⁻¹. The Ph, thousand grain weight, and grain yield values were corrected to 13% moisture.

The data was subjected to homogeneity of variance tests and, once the normality of the errors had been confirmed, analysis of variance was carried out using the F test. If significant, the Scott-Knott test and the T-test were applied, respectively, with p ≤ 0.05.

Results and Discussion

There was an interaction between the factors tested (2,4-D salts × cultivars × wheat application stages) for all the variables studied. The results show that the application of 2,4-D choline and amine salts caused phytotoxicity that varied according to the stage of development of the crop and also according to the cultivar used. At all stages of wheat development (before tillering, at full tillering, at the first node, and booting) there was phytotoxicity when the salts of 2,4-D (choline and amine) were applied compared to the control

Table 2. Climatic conditions at the time of application of 2,4-D salts to different wheat cultivars and crop development stages. UFFS/Erechim/RS, Brazil.

| Weather conditions | Wheat development stages | | | |
|----------------------------------|--------------------------|-------------------|-------------------|------------|
| | Before tillering | At full tillering | At the first node | Booting |
| Date of application | 28/06/2019 | 10/07/2019 | 09/08/2019 | 26/08/2019 |
| Air temperature (°C) | 24.8 | 24.2 | 18.3 | 21.3 |
| Soil temperature (°C) | 21.7 | 13.8 | 11.3 | 19.3 |
| Wind speed (km h ⁻¹) | 7.0 | 3.5 | 7.5 | 4.9 |
| Solar radiation (%) | 100 | 100 | 100 | 100 |

without the use of herbicides, except at budding at 7, 21, 28, and 35 DAT for cultivar OR 1403 and at this same phenological stage for cultivar TBIO Toruk at 21, 28, and 35 DAT (Table 3). This is possibly due to the fact that cultivars at this stage have more lignified tissues and are therefore more tolerant to the herbicide, which is absorbed in lower concentrations. Kumar & Singh (2010) report that 2,4-D is more efficient at translocating in plants with high metabolic activity, with plants gaining greater tolerance as they develop and get older.

Even though phytotoxicity occurred at all the times evaluated (7, 14, 21, 28, and 35 DAT), it can be considered low, as the highest level observed was 15% for the two 2,4-D salts when applied to the OR 1403 and TBIO Toruk cultivars. Assunção et al. (2017) when testing doses of flumioxazin applied to the wheat cultivar TBIO Sintonia reported the occurrence of phytotoxicity of up to 34% to the crop, however over time the plants were able to metabolize the herbicide with no negative effect on the thousand grain weight, hectolitre weight, and grain yield.

Cultivar OR 1403 showed greater phytotoxicity at 7, 14, 21, 28, and 35 DAT at the phenological stage of the first

node when the two salts of 2,4-D, choline and amine salt, were applied (Table 3). Greater phytotoxicity was observed when applying the two salts of 2,4-D (choline and amine) from 7 to 21 DAT at the first node stage for the cultivar TBIO Toruk and from 28 to 25 DAT the greatest effects of these herbicides were observed when using the products before tillering. The two wheat cultivars (OR 1403 and TBIO Toruk) showed the lowest phytotoxicity in all the periods (7, 14, 21, 28, and 35 DAT) in which the use of 2,4-D salts was evaluated at the rubbery phenological stage, due to the fact that wheat plants are more tolerant to herbicides, as explained above.

The results show that the application of 2,4-D choline and amine salt when applied to the first node caused greater phytotoxicity to cultivar OR 1403 when compared to TBIO Toruk (Table 3). This is due to the unique and different genetic characteristics that the cultivars have, such as size, tillering capacity, leaf index and area, root volume, the ability to metabolize herbicides, among others, that the materials can present in relation to the application of herbicides (Piasecki et al., 2017; Soligo et al., 2022). The OR 1403 cultivar is of

Table 3. Phytotoxicity (%) observed in wheat as a result of the application of 2,4-D salts (choline and amine), cultivars OR 1403 and TBIO Toruk and phenological stages of the crop, before tillering (BT), at full tillering (FT), at the first node (FN), and booting (BO). UFFS/Erechim/RS, Brazil.

| Treatments | Phytotoxicity to wheat (%) | | | | | | | |
|-------------------------------|----------------------------|----------|----------|----------|------------|---------|---------|--------|
| | OR 1403 | | | | TBIO Toruk | | | |
| | BT | FT | FN | BO | BT | FT | FN | BO |
| 7 Days after treatment (DAT) | | | | | | | | |
| Control | ns0.0 Ac ² | ns0.0 Ab | ns0.0 Ab | ns0.0 Aa | 0.0 Ac | 0.0 Ab | 0.0 Ab | 0.0 Ab |
| Choline salt | ns5.0 Cb | *8.8 Ba | *10.8 Aa | *0.0 Da | 5.0 Bb | 6.5 Ba | 8.8 Aa | 6.5 Ba |
| Amine salt | *6.5 Ba | *8.0 Ba | *10.8 Aa | *0.0 Ca | 7.0 Aa | 5.3 Ba | 8.0 Aa | 5.0 Ba |
| Overall average | 4.2 | | | | | | | |
| CV (%) | 20.6 | | | | | | | |
| 14 Days after treatment (DAT) | | | | | | | | |
| Control | ns0.0 Ac | ns0.0 Ac | ns0.0 Ab | ns0.0 Ac | 0.0 Ab | 0.0 Ab | 0.0 Ac | 0.0 Ab |
| Choline salt | *4.0 Bb | ns3.0 Bb | *15.0 Aa | ns4.0 Bb | 6.5 Aa | 4.0 Ba | 8.0 Ab | 4.0 Ba |
| Amine salt | ns6.5 Ba | ns5.0 Ba | *14.0 Aa | *6.5 Ba | 6.5 Ba | 5.0 Ca | 10.8 Aa | 4.0 Ca |
| Overall average | 4.5 | | | | | | | |
| CV (%) | 21.3 | | | | | | | |
| 21 Days after treatment (DAT) | | | | | | | | |
| Control | ns0.0 Ac | ns0.0 Ac | ns0.0 Ac | ns0.0 Aa | 0.0 Ac | 0.0 Ac | 0.0 Ac | 0.0 Aa |
| Choline salt | *8.0 Ba | *8.0 Bb | *13.5Ab | ns0.0 Ca | 6.5 Aa | 6.5 Ab | 5.0 Ab | 0.0 Ba |
| Amine salt | ns6.0 Cb | *10.0 Ba | *15.0 Aa | ns0.0 Da | 5.0 Bb | 8.8 Aa | 9.0 Aa | 0.0 Ca |
| Overall average | 4.2 | | | | | | | |
| CV (%) | 20.5 | | | | | | | |
| 28 Days after treatment (DAT) | | | | | | | | |
| Control | ns0.0 Ab | ns0.0 Ac | ns0.0 bA | ns0.0 Aa | 0.0 Ac | 0.0 Ac | 0.0 Ab | 0.0 Aa |
| Choline salt | ns8.0 Ba | ns8.0 Bb | *10.0 Aa | ns0.0 Ca | 8.0 Aa | 8.0 Ab | 4.0 Ba | 0.0 Ca |
| Amine salt | *8.0 Ba | *9.3 Aa | *10.0 Aa | ns0.0 Ca | 6.8 Bb | 11.3 Aa | 3.8 Ca | 0.0 Da |
| Overall average | 3.9 | | | | | | | |
| CV (%) | 15.9 | | | | | | | |
| 35 Days after treatment (DAT) | | | | | | | | |
| Control | ns0.0 Ac | ns0.0 Ac | ns0.0 Ac | ns0.0 Aa | 0.0 Ac | 0.0 Ac | 0.0 Ab | 0.0 Aa |
| Choline salt | *14.0 Aa | *9.0 Ca | *10.0 Bb | ns0.0 Da | 12.0 Aa | 8.0 Bb | 3.8 Ca | 0.0 Da |
| Amine salt | *11.8 Ab | *7.8 Bb | *11.3 Aa | ns0.0 Ca | 10.0 Ab | 10.0 Aa | 3.8 Ba | 0.0 Ca |
| Overall average | 4.6 | | | | | | | |
| CV (%) | 10.6 | | | | | | | |

ns or * Compares the two cultivars within 2,4-D salts and application times. ¹ Averages followed by the same uppercase letters in the row compare the application times within the 2,4-D salts for each cultivar. Averages followed by lowercase letters in the column compared the 2,4-D salts within each stage of wheat development for each cultivar.

medium height and has an early to medium cycle, while TBIO Toruk is short and has a medium ripening cycle, so there are some differences between the two. In addition, when working with these two cultivars, it was also hoped that they would show differences in terms of selectivity to 2,4-D salts, as observed in other studies in which these characteristics were evaluated with other wheat materials (Rodrigues et al., 2006; Rüdell et al., 2021).

Vargas & Roman (2005) also found low percentages of phytotoxicity for 2,4-D applications, where this was less than 5% in barley, white oats and ryegrass for all the seasons evaluated in the study. Piasecki et al. (2017) when evaluating the selectivity of herbicide combinations and doses in wheat post-emergence, observed that 2,4-D showed low phytotoxicity when used alone, regardless of the dose used, where levels were less than 5% at 14, 21, and 35 DAT. Viecelli et al. (2019), when studying the responses of wheat plants to combinations of herbicides with insecticides and fungicides, observed that the application of 2,4-D alone to wheat caused low percentages of phytotoxicity throughout the evaluations, corroborating the data found in this study.

In the tillering phase, the maximum phytotoxicity observed was 10% over the course of the evaluations for both cultivars, with the highest being for the application of 2,4-D amine salt, from 14 to 28 DAT (Table 3). Penckowski et al. (2003) observed that the maximum phytotoxicity caused by amine salt in the tillering phase of the wheat crop was 1.6% at 16 DAT, and at 30 DAT they no longer observed any injury symptoms. Roman et al. (2006) observed that applications of 2,4-D before tillering are considered very early and can cause phytotoxicity as well as retention of the ears in the stalk, a fact that was not observed in this study. Piasecki et al. (2017) when evaluating the phytotoxicity of herbicides applied for the management of weeds infesting wheat observed low damage when 2,4-D was applied, and after 35 DAT the damage symptoms were less than 5%. Rüdell et al. (2021) observed that the application of 2,4-D at the beginning of wheat tillering had a negative effect

on the crops yield parameters when compared to the use of iodosulfuron-methyl.

These different results found by the researchers may be linked to the different wheat cultivars used in the experiments, the formulations and types of herbicides, the climate and soil conditions, as well as the management and crop treatments adopted.

The 2,4-D salts differ in terms of droplet size and evaporation time, with the choline salt evaporating faster than the amine salt (Oliveira et al., 2019), which may be related to the phytotoxicity values found in the study. As the phytotoxicity values found for both salts were low, 2,4-D choline should be prioritized as it has greater stability and is less susceptible to volatilization than other 2,4-D formulations, as found in studies by Sosnoskie et al. (2015) and Peterson et al. (2016).

Phytotoxicity values vary due to the genetics involved in each cultivar, the age of the plant, as well as its ability to metabolize the herbicide, among others. Kumar & Singh (2010) report that plants have different resistance capacities depending on their age. During flowering, resistance to hormonal herbicides is reduced because the plant is in a state of high metabolic activity.

For the grain yield components (number of ears, length of ears, number of full and sterile grains, thousand grain weight, hectolitre weight, and grain yield), the OR 1403 and TBIO Toruk wheat cultivars were not compared to each other, as they are genetically different and if the analysis were carried out, the results could be masked, in other words, we wouldn't know if the differences in the results were due to the use of the herbicides (2,4-D salts) or the stage of application of the products or the genetic effect of the sown materials, which differ mainly in terms of plant cycle and height.

The results show that, for the two wheat cultivars (OR 1403 and TBIO Toruk), the best phenological stages for the application of 2,4-D choline salt were at full tillering, at the first node, and booting, where the highest number of ears of wheat was obtained (Table 4). The best phenological stages

Table 4. Number (m^{-2}) and length (cm) of ears in wheat as a function of the application of 2,4-D salts (choline and amine), cultivars (OR 1403 and TBIO Toruk) and phenological stages of the crop, before tillering (BT), at full tillering (FT), at the first node (FN), and booting (BO). UFFS/Erechim/RS, Brazil.

| Treatments | OR 1403 | | | | TBIO Toruk | | | |
|--------------------------|-----------------------|-----------|----------|----------|------------|-----------|-----------|-----------|
| | BT | FT | FN | BO | BT | FT | FN | BO |
| Number of ears (m^2) | | | | | | | | |
| Control | 472.2 Aa ¹ | 472.2 Aa | 472.2 Aa | 472.2 Aa | 396.7 Aa | 396.7 Aa | 396.7 Ab | 396.7 Aab |
| Choline salt | 348.0 Bc | 450.0 Aab | 460.0 Aa | 445.4 Aa | 348.0 Bb | 393.4 Aa | 405.4 Aab | 378.7 ABb |
| Amine salt | 404.0 Ab | 425.4 Ab | 341.4 Bb | 444.0 Aa | 349.4 Cb | 389.4 BCa | 441.4 Aa | 420.0 Aa |
| Overall average | 413.3 | | | | | | | |
| CV (%) | 5.3 | | | | | | | |
| Length of ears (cm) | | | | | | | | |
| Control | 8.8 Ab ¹ | 8.8 Ab | 8.8 Ab | 8.8 Aab | 7.9 Ac | 7.9 Ab | 7.9 Ab | 7.9 Aa |
| Choline salt | 9.8 Aa | 9.1 Bab | 9.4 Ba | 8.6 Cb | 8.7 Aa | 7.5 Cc | 8.6 Aa | 7.9 Ba |
| Amine salt | 9.9 Aa | 9.3 BCa | 9.4 Ba | 9.0 Ca | 8.4 Ab | 8.3 Aa | 7.7 Bb | 8.1 Aa |
| Overall average | 8.6 | | | | | | | |
| CV (%) | 1.9 | | | | | | | |

¹Averages followed by the same uppercase letters in the row compare the phenological stages at which the treatments were applied to each cultivar. Averages followed by lowercase letters in the column compared the treatments within each phenological stage of the cultivar.

for applying amine salt to the OR 1403 cultivar are: before tillering, at full tillering, and booting. As for the TBIO Toruk cultivar, the best results were obtained for the number of ears at the first node and booting stage.

When comparing the two salts with each other, it was observed that choline showed a greater number of ears at the first node stage and at booting for cultivar OR 1403, and amine at full tillering, first node, and booting for TBIO Toruk (Table 4). This may be linked to the levels of phytotoxicity caused by the herbicides at these respective stages, as well as the inhibition of gibberellin synthesis caused by the application of the herbicides, which caused the plant to decrease in height and develop other parts, such as the number of ears (Cenci et al., 2013). Some studies found no differences when applying 2,4-D at different doses and phenological stages for the number of ears of wheat (Rodrigues et al., 2006; Soligo et al., 2022). This is probably due to the use of different cultivars, doses of the herbicide, the location of the trials or even different climate and soil conditions.

The application of the two salts of 2,4-D before tillering of the two wheat cultivars was the one that showed the greatest ear length when comparing both the phenological stages within each treatment, as well as the herbicides within each crop stage (Table 4). The use of the two 2,4-D salts at the tillering stage onwards caused shorter cob lengths in the two cultivars (OR 1403 and TBIO Toruk) when compared to the application of the products at the phenological stage before tillering.

The application of 2,4-D amine at different stages (before tillering, at full tillering, at the emergence of the first visible node, and booting) and doses on the ORS Vintecinco wheat cultivar had no effect on the length of the crops ears, which is attributed to the metabolization of the herbicide by the plants (Soligo et al., 2022). The different responses to ear length in wheat are due to the various factors that can influence this variable, such as the cultivar sown, sowing density which, due to the number of plants per area, alters the length or even

environmental issues (Kumar & Singh, 2010; Soligo et al., 2022) and not just the effect of a particular herbicide. Galon et al. (2021) applied various herbicides to the TBIO Sinuelo wheat cultivar, including 2,4-D amine, and found no significant effect of the products used on the length of the crops ears. The herbicide 2,4-D can show different responses, depending on the time, dose and formulation in which it is applied (Kumar & Singh, 2010), thus justifying the variation in responses to ear length found in the studies.

It was observed that the number of full grains for the cultivars OR 1403 and TBIO Toruk were higher or equal when the 2,4-D choline and amine salt was applied at all the phenological stages in which they were used, compared to the control without herbicide application (Table 5). All the treatments (control without application, choline, and amine salt) showed no differences for any of the phenological stages in which they were used on the OR 1403 cultivar. And the use of the two 2,4-D salts before tillering of the TBIO Toruk cultivar showed a higher number of full grains than the applications made at the other stages of wheat development, which may be linked to the hormonal effect that these salts had on the plants.

The application of choline salt to the cultivar TBIO Toruk in the tillering phase was the only one to show a reduction in the number of full grains, matching the control and being inferior to 2,4-D amine (Table 5). Both salts showed a higher number of grains than the control for both cultivars, with the exception of the tillering stage for the choline salt, both for the OR 1403 and TBIO Toruk cultivars. This result may be related to the fact that salts cause stress in plants, causing them to develop certain parts, such as the greater number of full grains (Bernat et al., 2018).

With regard to the number of sterile grains, the results show that the two salts of 2,4-D applied at the different stages of development of the OR 1403 cultivar were either superior to the control without the use of herbicides, or equal to it, but never inferior (Table 5). When comparing the phenological

Table 5. Number of full and sterile grains in wheat as a function of the application of 2,4-D salts (choline and amine), cultivars (OR 1403 and TBIO Toruk) and phenological stages of the crop, before tillering (BT), at full tillering (FT), at the first node (FN), and booting (BO). UFFS/Erechim/RS, Brazil.

| Treatments | OR 1403 | | | | TBIO Toruk | | | |
|--------------------------|----------------------|---------|---------|---------|------------|---------|---------|---------|
| | BT | FT | FN | BO | BT | FT | FN | BO |
| Number of full grains | | | | | | | | |
| Control | 40.8 Aa ¹ | 40.8 Ab | 40.8 Aa | 40.8 Aa | 36.6 Ab | 36.6 Ab | 36.6 Ab | 36.6 Aa |
| Choline salt | 42.3 Aa | 41.3 Ab | 40.8 Aa | 41.8 Aa | 42.5 Aa | 35.9 Bb | 40.9 Aa | 37.1 Ba |
| Amine salt | 42.3 Aa | 44.0 Aa | 42.5 Aa | 42.5 Aa | 40.5 Aa | 40.8 Aa | 36.9 Bb | 37.8 Ba |
| Overall average | 39.9 | | | | | | | |
| CV (%) | 3.6 | | | | | | | |
| Number of sterile grains | | | | | | | | |
| Control | 2.5 Ab | 2.5 Aa | 2.5 Aab | 2.5 Aa | 3.7 Aa | 3.7 Aa | 3.7 Aa | 3.7 Aa |
| Choline salt | 4.4 Aa | 2.9 BCa | 3.3 Ba | 2.2 Ca | 3.1 Aa | 3.7 Aa | 2.9 Aa | 3.7 Aa |
| Amine salt | 3.9 Aa | 2.3 Ba | 2.2 Bb | 2.7 Ba | 3.7 Aa | 3.6 Aa | 2.9 Aa | 2.8 Aa |
| Overall average | 3.1 | | | | | | | |
| CV (%) | 17.3 | | | | | | | |

¹Averages followed by the same uppercase letters in the row compare the phenological stages at which the treatments were applied to each cultivar. Averages followed by lowercase letters in the column compared the treatments within each phenological stage of the cultivar.

stages for each treatment, it was observed that the use of 2,4-D choline and amine at an early stage (before tillering) caused the highest number of sterile grains in the OR 1403 wheat cultivar. For the cultivar TBIO Toruk, there was no difference when applying the treatments within each phenological stage or even between the phenological stages compared to each other, for each individual treatment. This is probably due to the different characteristics of the two wheat cultivars, which respond differently to herbicide application.

The number of full or sterile grains can be altered by some stress during plant growth, such as the use of a herbicide, time of application or dose, type of soil, weather conditions, and the plant itself, or even depending on the cultivar sown (Deboer et al., 2011; Rüdell et al., 2021). Ionescu & Penescu (2015), when evaluating aspects of the physiology of winter wheat treated with herbicides, reported that 2,4-D, being an auxin mimic, when it comes into contact with the plant, can interrupt the inducing signals and generate side effects, including localized abnormal growth, twisting of leaves, stems, curving of shoots, among others, and can also cause the deposition of nutrients in the grains.

The thousand grain weight of wheat showed that the use of 2,4-D salts was either equal to the control without herbicide application or superior for both cultivars when evaluating the effect of the treatments within each phenological stage (Table 6). There was little difference between the treatments, even though they were applied at different times in the crops development. For the OR 1403 cultivar, the best stage to apply the two salts of 2,4-D is before tillering and for TBIO Toruk, the same stage and also at the time of the booting obtained the highest thousand grain weight. In the other stages (at full tillering, first node, and booting) for cultivar OR 1403 and in at

full tillering and first node for TBIO Toruk, it was observed that choline and amine salt should not be applied, as they showed lower thousand grain weight when compared to the other stages for the two wheat cultivars. This is probably due to the fact that when herbicides are applied during these phases, they interfere with the plants metabolism, leading wheat to invest less in photoassimilates for grain filling and weight.

Vicelli et al. (2019) found no reduction in the thousand grain weight of wheat when it received an isolated application of 2,4-D during the third tiller stage. Soligo et al. (2022) observed no differences in the weight of a thousand grains of wheat when they applied different doses of 2,4-D before tillering, at full tillering, at the first node, and booting of the crop. Rüdell et al. (2021) when applying different herbicides to wheat observed that 2,4-D amine had negative effects on the productive parameters (number of tillers, number of ears, thousand grain weight, and grain yield) of the wheat TBIO Toruk and TBIO Sinuelo cultivars. The differences in responses to the use of 2,4-D in wheat in the studies are probably related to the type and formulation of herbicide used, the cultivar or even the stage at which the herbicides were applied.

The hectolitre weight was higher when using the 2,4-D amine salt for all the development stages in which this herbicide was applied, both for cultivar OR 1403 and TBIO Toruk, when compared to the choline salt and the control without the use of products (Table 6). When comparing the stages within each treatment, it was observed that the application of the two herbicides (choline and amine salts) were better, with a higher hectolitre weight at the beginning of the wheat cultivar OR 1403. For the cultivar TBIO Toruk, no differences were observed between the stages of application of the 2,4-D choline salt for the hectolitre weight of the

Table 6. Thousand grain weight (g), hectolitre weight (kg hl⁻¹), and grain yield of wheat as a function of the application of 2,4-D salts (choline and amine), cultivars (OR 1403 and TBIO Toruk) and phenological stages of the crop, before tillering (BT), at full tillering (FT), at the first node (FN), and booting (BO). UFFS/Erechim/RS, Brazil.

| Treatments | OR 1403 | | | | TBIO Toruk | | | |
|---|----------------------|---------|----------|---------|------------|---------|----------|---------|
| | BT | FT | FN | BO | BT | FT | FN | BO |
| Thousand grain weight (g) | | | | | | | | |
| Control | 32.4 Ab ¹ | 32.4 Aa | 32.4 Aa | 32.4 Aa | 35.5Ab | 35.5 Aa | 35.5 Aa | 35.5 Ab |
| Choline salt | 36.7 Aa | 30.6 Ba | 31.0 Ba | 30.7 Ba | 38.2 Aa | 34.5 Ba | 34.2 Ba | 39.2 Aa |
| Amine salt | 35.3 Ab | 31.4 Ba | 30.6 Ba | 31.5 Ba | 39.2 Aa | 35.4 Ba | 34.6 Ba | 39.4 Aa |
| Overall average | 34.3 | | | | | | | |
| CV (%) | 3.6 | | | | | | | |
| Hectolitre weight (kg hl ⁻¹) | | | | | | | | |
| Control | 72.8Aab | 72.8 Ab | 72.8 Ab | 72.8 Ab | 73.3 Aa | 73.3 Ab | 73.3 Aa | 73.3 Aa |
| Choline salt | 71.7 Bb | 71.4 Bc | 74.1 Aa | 73.5 Ab | 71.9 Ab | 72.4 Ab | 71.8Ab | 73.0 Aa |
| Amine salt | 73.1 Ba | 74.4 Ba | 73.7 Bab | 75.9 Aa | 73.3 Ba | 75.7 Aa | 74.1 Ba | 73.2 Ba |
| Overall average | 73.2 | | | | | | | |
| CV (%) | 1.0 | | | | | | | |
| Grain yield of wheat (kg ha ⁻¹) | | | | | | | | |
| Control | 3482 Aa | 3482 Bb | 3482 Ab | 3482 Ab | 3330 Aa | 3330 Aa | 3330 Aab | 3330 Ab |
| Choline salt | 2755 Cb | 3629 Ab | 3755 Aa | 3080 Bc | 2535 Cb | 3262 Aa | 3439 Aa | 2877 Bc |
| Amine salt | 2652 Cb | 4173 Aa | 3110 Bc | 3925 Aa | 2007 Cc | 3376 Ba | 3158 Bb | 3779 Aa |
| Overall average | 3282 | | | | | | | |
| CV (%) | 4.9 | | | | | | | |

¹Averages followed by the same uppercase letters in the row compare the phenological stages at which the treatments were applied to each cultivar. Averages followed by lowercase letters in the column compared the treatments within each phenological stage of the cultivar.

wheat. For the 2,4-D amine salt, the best hectolitre weight was observed when the herbicide was applied at full tillering, as its effect favoured the amount of mass produced in the grain. There was no effect on hectolitre weight from the use of doses of 2,4-D amine applied at different stages of wheat development in a study by [Soligo et al. \(2022\)](#). [Piasecki et al. \(2017\)](#) also found no differences in hectolitre weight when assessing the selectivity of 2,4 D and other herbicides applied to the Iguaçú wheat cultivar. These results may be related to the characteristics of the cultivar used, the doses and formulation of the herbicide, as well as the soil and weather conditions, as these are factors that affect the hectolitre weight of the wheat.

The results show higher wheat grain yields when 2,4-D amine was applied to the wheat cultivars OR 1403 and TBIO Toruk, at the tillering and booting phenological stages of the crop ([Table 6](#)). The highest grain yields were observed for 2,4-D choline when it was applied to the OR 1403 cultivar at the first node phenological stage, even higher than 2,4-D amine and the control without herbicides. [Soligo et al. \(2022\)](#) observed higher wheat grain yields when using lower doses of 2,4-D amine at full tillering and at the first node compared to other phenological stages of the crop. The same authors also report that very early applications, such as before tillering, and very late applications, such as during booting, caused a decrease in wheat grain productivity. Similar results were found by [Tottman \(1977\)](#) where the very early application of 2,4-D and MCPA affected the morphology of the plant, causing alterations to the leaves and ears, interfering with the distribution of new leaves and also the beginnings of spikelets.

The use of herbicides on the TBIO Toruk cultivar showed higher grain yields when choline salt was applied at full tillering and first node stages, with greater or equal yields when compared to 2,4-D amine ([Table 6](#)). The TBIO Toruk cultivar showed higher yields when 2,4-D amine was used at the booting phenological stage, with higher results than 2,4-D choline and the control without application.

The results show the lowest grain yields for the OR 1403 and TBIO Toruk cultivars when the 2,4-D choline and amine salts were applied at the phenological stage before wheat tillering, with lower values than all the other crop development stages and in relation to the control without herbicide application ([Table 6](#)). There was a 22.4% (778.3 kg) and 31.8% (1,059.4 kg) reduction in the grain yield of wheat cultivars OR 1403 and TBIO Toruk when comparing the average use of 2,4-D choline and amine against the control without herbicides, respectively. This reduction in grain yield may be linked to the greater phytotoxicity observed at 35 DAT for both cultivars when the 2,4-D salts were applied before tillering ([Table 4](#)), i.e. the crop was unable to rid itself of the toxic effects of the herbicides over time, reducing grain yield as a result. The reduction in wheat yield after the application of 2,4-D may be linked to the effect of auxin on cell division, elongation and vascular differentiation, which in adequate doses can stimulate these processes, but when in high concentrations

affects plant growth processes and causes negative damage to the crop ([Kumar & Singh, 2010](#)).

In the literature there is a contradiction between the results related to the use of 2,4-D on different wheat cultivars. [Soligo et al. \(2022\)](#) observed a linear reduction in the grain yield of the OR Vintecincio wheat cultivar as the doses of the herbicide increased, when applied before tillering, at full tillering, first node, and booting of the crop. The application of different doses of 2,4 D to wheat did not cause a reduction in crop yield when compared to the control ([Piasecki et al., 2017](#); [Viecelli et al., 2019](#)). The contradiction between the research results could probably be related to the cultivar used, the phase applied, the soil and climate conditions, or even the doses and formulation of the product.

Conclusions

The stage of crop development, the cultivar used and the type of 2,4-D salt applied to the wheat caused variations in phytotoxicity and the effects on the crops grain yield components.

The salts of 2,4-D, choline and amine cause greater phytotoxicity when applied at the first node phenological stage of the OR 1403 cultivar, and comparatively less in the TBIO Toruk cultivar.

In general, the highest number of ears, ear length, number of full grains, thousand grain weight, hectolitre weight, grain yield, and lowest number of sterile grains occurred when the 2,4-D choline salt was applied at the first stage of the OR 1403 cultivar.

In general, the highest number of ears, ear length, number of full grains, thousand grain weight, hectolitre weight, grain yield, and the lowest number of sterile grains occurred when the 2,4-D amine salt was applied to the TBIO Toruk cultivar at the booting stage.

The highest grain yields were observed when 2,4-D choline and amine were applied to the wheat cultivars OR 1403 and TBIO Toruk at the booting phenological stage of the crop.

The use of 2,4-D choline and amine caused the lowest grain yields when applied to the wheat cultivars OR 1403 and TBIO Toruk at a phenological stage before tillering of the crop.

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Compliance with Ethical Standards

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AJC, LG, EROR, FACS, RJT, GFP; Methodology: AJC, LG, GFP; Project administration: AJC, LG, GFP; Supervision: LG, GFP; Validation: AJC, LG, EROR, FACS, RJT, GFP; Visualization: AJC, LG, EROR, FACS, RJT, GFP; Writing – original draft: AJC, LG, EROR, FACS, RJT, GFP; Writing – review & editing: AJC, LG, EROR, FACS, GFP.

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