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# Selectivity of 2,4-D salts to barley plants applied at distinct phenological crop stages

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**ABSTRACT:** The herbicide 2,4-D is available in distinct formulations in terms of selectivity to non-target species, and in terms of drift and volatilization potentials. Thus, the objective of this work was to evaluate the selectivity and effect of 2,4-D formulations (amine salt and choline) applied at different developmental stages of barley. The experiment was installed in the field in randomized blocks, in a 2 × 4 + 1 factorial scheme, with four replications. Factor A comprised the formulations of 2,4-D (amine salt and choline), and factor B the crop phenological stage (before tillering start, at full tillering, first node and booting), plus a weeded control treatment. The 2,4-D amine showed higher phytotoxicity when applied before tillering and at the first node. On the other hand, 2,4-D choline, when used prior to tillering start, showed the highest phytotoxicities. The yield components showed positive results when using the amine salt before and at tillering, while in the first node stage, and in booting, the choline salt stood out. The use of 2,4-D amine in tillering and choline in the first node provided the highest grain yield of barley.

**Key words:** auxin-mimic herbicides; *Hordeum vulgare*; phenological stages

# Seletividade de sais de 2,4-D aplicados em diferentes estádios fenológicos da cevada

**RESUMO:** O herbicida 2,4-D está disponível em algumas formulações que apresentam diferenças em relação à seletividade das espécies em que são usados e quanto aos efeitos de deriva e volatilização. Assim, objetivou-se com o trabalho avaliar a seletividade e o efeito de formulações de 2,4-D (sal amina e colina) aplicados em diferentes estádios de desenvolvimento da cevada. O experimento foi instalado a campo em blocos casualizados, no esquema fatorial 2 × 4 + 1, com quatro repetições. No fator A alocou-se as formulações de 2,4-D (sal amina e colina) e no B as épocas de aplicação (antes do perfilhamento, no perfilhamento, primeiro nó e após primeiro nó), mais uma testemunha capinada. O 2,4-D amina apresentou maior fitotoxicidade quando aplicado antes do perfilhamento e no primeiro nó. Já o 2,4-D colina na fase anterior ao perfilhamento demonstrou as maiores fitotoxicidades. Os componentes de rendimento apresentaram resultados positivos ao se usar o sal amina antes e no perfilhamento, enquanto que no primeiro nó e após primeiro nó, o sal colina sobressaiu-se. O 2,4-D amina e colina aplicados no perfilhamento e no primeiro nó, respectivamente, proporcionaram a maior produtividade de grãos da cevada.

**Palavras-chave:** herbicidas auxínicos; *Hordeum vulgare*; estádio de aplicação

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## Introduction

In Brazil, in the 2020/21 crop year, barley (*Hordeum vulgare* L.) culture occupied an area of 111.75 thousand hectares, with average productivity of 3.81 t $ha^{-1}$, totaling a production of 425.76 thousand tons, representing a 12% increase over the previous crop year (CONAB, 2022). Despite the increase in the cultivated area and in barley production in recent years, the average Brazilian productivity is far below that obtained in crops that adopt a high level of technology or in demonstration areas. Among the main factors affecting barley grain yield and quality are the cultivar used, soil fertility, and management of diseases, insects, and weeds.

When not properly controlled, weeds can cause high losses to producers by competing with the crop for water, light, and nutrients. In addition, weeds can host insects and diseases, and also release allelopathic substances that interfere with barley growth, development, and consequently grain yield and quality (Tironi et al., 2014; Karpinski et al., 2018; Galon et al., 2022).

The 'nabo' (*Raphanus raphanistrum*), 'nabiça' (*R. sativus*), 'buvas' (*Conyza bonariensis*, *C. canadensis*, and *C. sumatrensis*), 'serralha' (*Sonchus oleraceus*), 'flor-roxa' (*Echium plantagineum*), and 'cipó-de-veado' (*Polygnum convolvulus*) are the main weed species of the Eudicot class that can infest winter-sown crops (Tironi et al, 2014; Roso et al., 2017; Schneider et al., 2021). In a recent study it was observed that the presence of 'nabo' can affect grain yield and cause economic damage in barley when not controlled until 22 days after crop emergence (Galon et al., 2022).

In addition to high competitive ability, a major concern with infestation of 'nabo', 'nabiça', and 'buvas' in winter crops is due to shortages of herbicides registered for the control of these weeds as they infest barley (Galon et al., 2022; MAPA, 2022). The 'nabo' and 'nabiça' rape appear in a dominant way in the winter sown crops in the South Region of Brazil, mainly because they are used as pasture or even as a mulch plant (after the soybean harvest until the start of wheat sowing). The use of these species also occurs for the adoption of the no-till farming system, promoting an increase in the soil's seed bank. Moreover, in Brazil, cases of resistance of 'nabo' or 'nabiça' plants to herbicides belonging to the mechanism of action of the aceto lactate synthetase enzyme inhibitors - ALS - have already been reported (Cechin et al., 2016), which makes it even more difficult to adopt chemical control in barley culture.

The 'buva' species of the genus *Conyza* (*C. bonariensis*, *C. canadensis*, and *C. sumatrensis*) stand out among the most problematic, harmful, invasive and widely distributed in agriculture worldwide (Bajwa et al., 2016). In Brazil, 'buva' has infested both crops sown in winter and those grown in summer, requiring year-round management of these species (Piasecki et al., 2019). This weed has been described as resistant to herbicides that inhibit the enzymes 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) and ALS (Heap, 2022). These herbicides are commonly used for desiccation of the area, limiting their use in subsequent weed control and thus restricting the number of products available as a possible alternative to chemical management.

Eudicotyledonous weed control in barley cultivation can be effected with the use of 2,4-dichlorophenoxyacetic acid, better known as 2,4-D. This herbicide is characterized by high efficacy, low cost, selectivity on grasses, and is an option for controlling plants that are resistant to other mechanisms of action, such as 'nabo', 'nabiça', 'flor-roxa', or even species of 'buva' (Cechin et al., 2016; MAPA, 2022).

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 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 for weed control can cause injury to cereal crops such as barley. These effects are mainly related to the formulation and dose of the product, in addition to the crop development stage and soil and climate conditions at the time of application (Kumar & Singh, 2010; Peterson et al., 2016; Skelton et al., 2017).

Among the direct and indirect effects already described by the use of 2,4-D, phytotoxicity, altered nutrient uptake, downregulation of plant defense mechanisms, and reduced grain yield components stand out (Galon et al., 2014; Piasecki et al., 2017; Skelton et al., 2017). 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 higher water solubility and adsorption to soil and, lower volatility when compared to ester formulation (Roman et al., 2006). Also, the amine salt requires a shorter time to cross the leaf cuticle compared to the other salts (Contiero et al., 2016). The 2,4-D salt choline formulation features Colex-D technology and has as a distinguishing feature the reduced percentage of finer droplets, ultra-low volatility, and reduced odor when compared to the other formulations (Sosnoskie et al., 2015; Skelton et al., 2017). The lower volatility of 2,4-D choline, however, may influence its retention in leaves and promote foliar injury depending on the stage of crop development (Oliveira et al., 2019).

Studies of 2,4-D formulations are of utmost importance for better understanding regarding its efficacy, speed, control spectrum, selectivity, and toxicity in different crops (Kumar & Singh, 2010; Marcinkowska et al., 2017). The selectivity of auxin mimicking herbicides to barley and other grasses, among them 2,4-D, is given by mechanisms such as low leaf penetration, limited translocation in phloem, insensitivity at the site of action and low metabolization of herbicides (Roman et al., 2006; Peterson et al., 2016). In addition, herbicide tolerance depends on some factors such as product formulation and applied dose, crop development stage, environmental and soil conditions (Roman et al., 2006; Peterson et al., 2016).

Given the above, the hypothesis of the paper is that barley plants may show different responses in terms of selectivity and grain yield components when exposed to different salts of 2,4-D (amine or choline salt) at the developmental stages before tillering, at tillering, at the first node, and after the first node. Thus, the objective of this study was to evaluate the selectivity and the effect of 2,4-D amine and choline salt formulations applied at different stages of development of barley cultivar BRS Cauê.

## Materials and Methods

### Growing conditions and experimental design

The experiment was conducted in the field, in the experimental area of the Universidade Federal da Fronteira Sul (UFFS), Campus Erechim, RS, Brazil, from June to November 2019. According to Köppen classification, the regions climate is classified as fundamental type C, subtype fa, characterized as humid subtropical, with no defined dry season, with the temperature of the hottest month exceeding 22 °C, an average annual temperature of 18.2 °C, and average annual precipitation of 1,869 mm (CEMETRS, 2012). The site is located in the Alto Uruguay physiographic region of Rio Grande do Sul, Brazil, at an altitude of 760 m.

The soil of the area is characterized as typical Red Aluminoferric Latosol (Santos et al., 2018), and has the following physicochemical characteristics: pH in water = 4.7; OM = 3.04%; P = 8.5 mg dm$^{-3}$; K = 106 mg dm$^{-3}$; $Al^{3+}$ = 1.0 cmol$_c$ dm$^{-3}$; $Ca^{2+}$ = 5.1 cmol$_c$ dm$^{-3}$; $Mg^{2+}$ = 3.4 cmol$_c$ dm$^{-3}$; CTC$_{efetive}$ = 9.9 cmol$_c$ dm$^{-3}$; CTC$_{pH7}$ = 18.6 cmol$_c$ dm$^{-3}$; H + Al = 9.7 cmol$_c$ dm$^{-3}$; base saturation = 48%; and, clay = 64%. Fertility correction was carried out according to the needs observed in the soil analysis and according to the liming and fertilization recommendations for the barley crop (ROLAS, 2016).

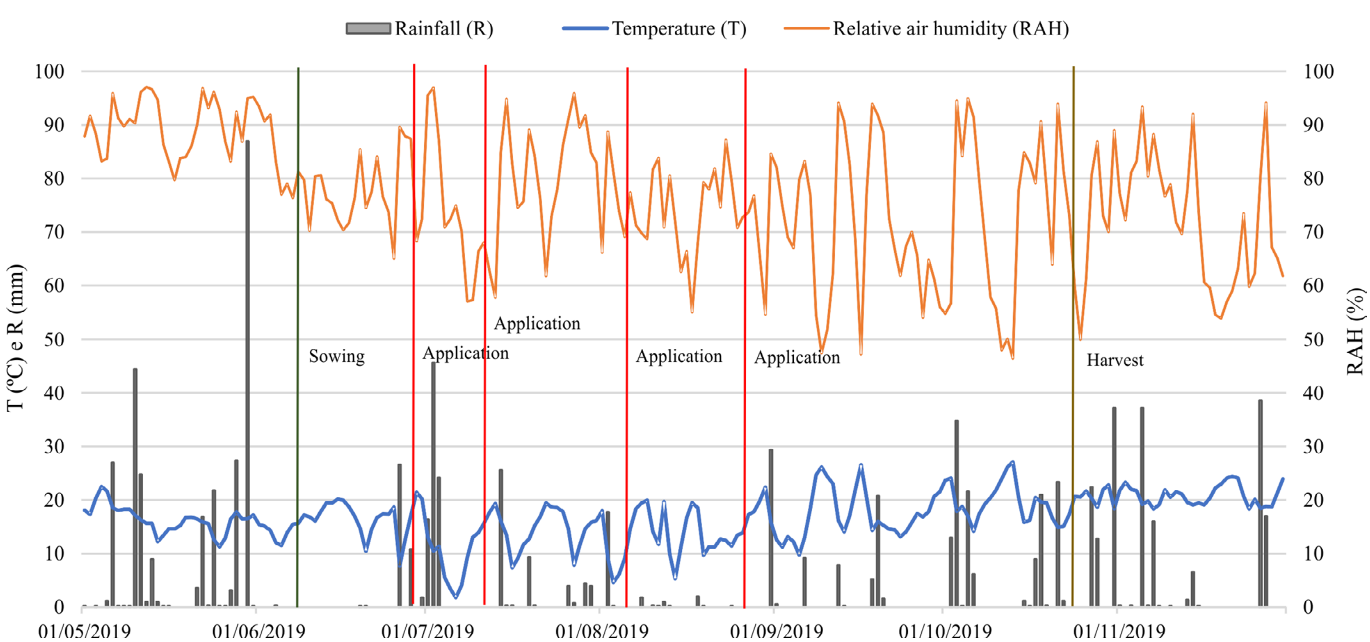
The sowing of the barley cultivar BRS Cauê was performed in a no-till system, and 15 days before this operation the vegetation was desiccated with glyphosate herbicide at a dose of 1,080 g ha$^{-1}$ of acid equivalent.

The trial was set up in a randomized block design, arranged in a 2 × 4 + 1 factorial scheme, with four repetitions. Factor A allocated the salts of 2,4-D (amine salt and choline salt) and factor B allocated the herbicide applications at different stages of barley development (before tillering, at tillering, at the first node, and after the first node), plus a weeded witness. Each experimental unit was composed of a 13.6 m² area, 2.72 m wide by 5 m long, where 16 barley lines, spaced at 0.17 m apart, were sown on 10/06/2019, with a depth of 0.1-0.2 m and a density of 300 plants m$^{-2}$. The usable area of the plots corresponded to the 10 central rows, discarding 3 rows on each side and 0.5 m from the front edges.

### Application of treatments

The herbicides 2,4-D amine and 2,4-D choline were applied at a dose of 670 g ha$^{-1}$ acid equivalent, which resulted in the application of 1.0 and 1.46 L ha$^{-1}$ of DMA® 806 BR (2,4-D amine) and Enlist® Colex-D (2,4-D choline), respectively. Although the two herbicides studied (DMA® 806 BR and Enlist® Colex-D) are not registered for application in barley (MAPA, 2022), many producers have been using them to control Eudicotyledonous weeds in this crop, especially ‘nabo’, ‘nabiça’, ‘flor-roxa’, and ‘buvas’ because these species are resistant to other products, as already reported.

The herbicides were applied using a $CO_2$ pressurized precision knapsack sprayer equipped with four DG 110.02 fan spray tips under a constant pressure of 210 kPa and a travel speed of 3.6 km h$^{-1}$, with a flow rate of 150 L ha$^{-1}$ of solution. The herbicides were applied post-emergence to barley at different developmental stages (before tillering, at tillering,



Source: INMET (2019).

**Figure 1.** Average temperature (°C), rainfall (mm), and relative air humidity (%) during the period of the experiment conduction from June to November 2019. UFFS/Erechim/RS/Brazil, 2019.

**Table 1.** Application date, environmental conditions, and developmental stages of barley when applying 2,4-D salts (amine and choline). UFFS/Erechim/RS/Brazil, 2019.

| Date and environmental conditions | Stage of barley development at herbicide applications | | | |
|---|---|---|---|---|
| | Before tillering | At tillering | At the first node | After the first node |
| Date of application | 28/06/2019 | 10/07/2019 | 05/08/2019 | 26/08/2019 |
| Air temperature (°C) | 24.8 | 24.2 | 19.7 | 19.9 |
| Soil temperature (°C) | 21.7 | 15.8 | 13.1 | 17.9 |
| Luminosity (%) | 100 | 100 | 100 | 100 |
| Wind speed (km $ha^{-1}$) | 7.0 | 3.5 | 7.1 | 4.7 |
| Relative air humidity (%) | 55 | 56 | 58 | 58 |

at the first node, and after the first node) and environmental conditions as shown in Table 1.

The weeds that emerged and infested the experiment were eliminated from the experimental units mechanically (directed weeding) whenever necessary to avoid competition effects on the treatments.

### Toxicity

The visual evaluation of phytotoxicity was performed at 7, 14, 21, 28, and 35 days after the application of treatments (DAT), assigning percentage scores from zero (0%) to one hundred (100%), where 0% means no damage and 100% indicates the death of barley plants, according to the methodology proposed by SBCPD (1995).

### Grain yield components

The number of ears ($m^{-2}$) was determined at pre-harvest of the barley, in an area of 0.5 × 0.5 m in the central region of each experimental unit. To determine the length of ears (cm) and the number of full and sterile grains, 10 ears were randomly harvested in each experimental unit, packed in kraft paper bags and sent to the Sustainable Management of Agricultural Systems Laboratory of the UFFS, Campus Erechim, RS, Brazil, where the evaluations were performed.

After the barley was harvested by hand on a 5.1 m² surface area when it had 15% moisture, the hectoliter weight (kg $hl^{-1}$), the thousand-grain mass (g), and the grain yield (kg $ha^{-1}$) were determined. The PH was determined using a Dalle Molle scale, model 40. The mass of one thousand grains was measured by counting eight samples of 100 grains each and then weighed on an analytical balance. Grain yield was then estimated and extrapolated in kg $ha^{-1}$. For the analyses, the moisture content of the grains was adjusted to 13%.

### Statistical analysis

The data were submitted to variance analysis using the F test, and if significant, the Tukey test was applied at $p \leq 0.05$ to compare the means of the treatments.

## Results and Discussion

There was an interaction between the formulations of the amine and choline salts of 2,4-D and the developmental stages of the barley plants during the application of the treatments for the phytotoxicity and grain yield components evaluated (Tables 2, 3 and 4).

The use of 2,4-D amine caused an average phytotoxicity of 9.5% at 21 DAT, when applied before and during the tillering stage, and 10% at 35 DAT when sprayed at the first node (Table 2). The 2,4-D choline salt caused phytotoxicity around 8.5% from 7 to 35 DAT when applied prior to spraying. The same treatment used during tillering, first node and after first node caused an average phytotoxicity of less than 6.5%. In general, a low phytotoxicity was observed with the application of 2,4-D salts, not exceeding 10%.

Associated with the phytotoxicity symptoms was the occurrence of some twisted, abraded, or curled leaves, regardless of the development stage of the barley plants during the application of the herbicides. The application of 2,4-D amine (1,050 g $ha^{-1}$ active ingredient) before tillering and at tillering of barley cultivars MN 610 and Crioula demonstrated phytotoxicity contents, at 28 DAT, ranging from 3 to 15% and also the occurrence of twisted leaves (Galon et al., 2014). These symptoms occur due to the effect of synthetic auxin on the inducing signals, where the hormones, upon sensing the auxin present in the herbicide, interrupt the natural development signals, coming to cause leaf twisting, in addition to other deformations in the stem or roots (Ionescu & Penescu, 2015). The use of the herbicide 2,4-D amine (480 and 960 g $ha^{-1}$ acid equivalent) in post-emergence, of the barley cultivar BRS 225, when it presented 3 to 5 leaves did not cause injury when applied alone, thus being selective to the crop (Vargas & Roman, 2005). The results observed in the present study and those of other researchers indicate that the selectivity of 2,4-D on barley is dependent on the dose applied, the cultivar, and the stage of development of the crop, among others.

In addition, changes in leaf structures and/or phytotoxicity, caused by application stage or abiotic conditions, can affect the uptake, translocation, and metabolization of the herbicide 2,4-D (Peterson et al., 2016). Marcinkowska et al. (2017) observed that the precipitation of 2,4-D choline on leaves can form an amorphous deposit with the presence of crystals of the salt, thus causing spot injury to leaves.

Post-emergence application of the herbicide 2,4-D amine for barley weed management has caused phytotoxicity of less than 5% in barley plants (Vargas & Roman, 2005) and 10% in wheat crop (Piasecki et al., 2017). These studies corroborate in part with the results found in the present work.

The two salts of 2,4-D (amine and choline) caused the lowest number of ears when applied before tillering (Table 3)

**Table 2.** Phytotoxicity to barley cultivar BRS Cauê evaluated at 7, 14, 21, 28, and 35 days after treatment application (DAT), as a function of the use of 2,4-D amine and choline salts at different phenological stages of the crop: before tillering, at tillering, at the first node, and after the first node. UFFS/Erechim/RS/Brazil, 2019.

| Treatments | Periods of application | | | |
|---|---|---|---|---|
| | Before tillering | At tillering | At the first node | After the first node |
| | Phytotoxicity (%) at 7 DAT | | | |
| Without herbicide | 0.0 cA[1] | 0.0 bA | 0.0 cA | 0.0 bA |
| 2,4-D amine | 5.0 bB | 6.5 aB | 10.0 aA | 6.5 aB |
| 2,4-D choline | 8.0 aA | 5.0 aB | 6.5 abAB | 6.5 aAB |
| Overall average | 4.5 | | | |
| CV (%) | 21.3 | | | |
| | Phytotoxicity (%) at 14 DAT | | | |
| Without herbicide | 0.0 bA[1] | 0.0 bA | 0.0 cA | 0.0 bA |
| 2,4-D amine | 6.5 aB | 5.0 aB | 6.0 bB | 9.3 aA |
| 2,4-D choline | 6.0 aB | 5.0 aB | 9.0 aA | 8.7 aA |
| Overall average | 4.6 | | | |
| CV (%) | 20.1 | | | |
| | Phytotoxicity (%) at 21 DAT | | | |
| Without herbicide | 0.0 bA[1] | 0.0 cA | 0.0 bA | 0.0 aA |
| 2,4-D amine | 9.0 aA | 10.0 aA | 2.5 aB | 0.0 aC |
| 2,4-D choline | 9.0 aA | 8.0 bA | 3.0 aB | 0.0 aC |
| Overall average | 3.5 | | | |
| CV (%) | 28.0 | | | |
| | Phytotoxicity (%) at 28 DAT | | | |
| Without herbicide | 0.0 bA[1] | 0.0 bA | 0.0 bA | 0.0 aA |
| 2,4-D amine | 9.3 aA | 6.0 aA | 6.5 aA | 0.0 aB |
| 2,4-D choline | 9.0 aA | 6.5 aB | 5.0 aB | 0.0 aC |
| Overall average | 3.5 | | | |
| CV (%) | 27.0 | | | |
| | Phytotoxicity (%) at 35 DAT | | | |
| Without herbicide | 0.0 cA[1] | 0.0 bA | 0.0 cA | 0.0 bA |
| 2,4-D amine | 5.0 bB | 6.5 aB | 10.0 aA | 6.5 aB |
| 2,4-D choline | 8.0 aA | 5.0 aB | 6.5 bAB | 6.5 aAB |
| Overall average | 4.5 | | | |
| CV (%) | 21.32 | | | |

[1] Averages followed by lower case letters in the column and upper case letters in the row, do not differ by Tukey test (p ≥ 0.05).

and only 2,4-D choline was harmful to this variable studied in the tillering period and after the first node of barley plants, compared to plants without the application of herbicides. Although they found reduced percentage of phytotoxicity, Galon et al. (2014) also found reduced ears number when applying 2,4-D amine to barley cultivars MN610 and Crioula.

Regarding the use of herbicides in each stage of crop development, it was observed that the treated plants did not only show reductions in relation to the plants without herbicide. The application of 2,4-D amine caused a greater number of ears on barley plants after application during the tillering and first node stage, compared to application before tillering (Table 3). The 2,4-D choline, on the other hand, promoted a greater number of ears when applied at tillering stage and after the first node, compared to the treatment before tillering and at the first node. Stress situations during the vegetative period of plants can accelerate their reproductive development, as found by Bernat et al. (2018).

Barley ears length showed reduced values with the application of 2,4-D amine at the first node stage and after the first node of the crop, compared to the application of 2,4-D choline after the first node, compared to the other developmental stages of barley plants (Table 3). Early application of herbicides can cause the apex to be attached to the stalk by the ridges, which is responsible for the retention of ears in the thatch, as observed in wheat plants exposed to 2,4-D before tillering (Vargas & Roman, 2005), thus affecting the length of ears.

The application of 2,4-D amine showed a higher number of filled kernels only when used before tillering, compared to 2,4-D choline and the witness without herbicide (Table 3), which were reduced with the application of the herbicide at the other stages of development, both compared to the witness and to the application before tillering.

On the other hand, the use of 2,4-D amine promoted a higher number of full grains when applied at the first node, compared to plants in the control and 2,4-D choline treatments (Table 3). Herbicides, especially auxins can cause interference in the floral differentiation of crops, which contributes to having a higher number of grains in the case of the present study. Similar results were observed by Chao et al. (1994) where they found that 2,4-D amine reduced tillering in barley due to increased apical dominance and promoted greater number of full grains in the oat crop.

**Table 3.** Number of ears, length of ears, number of full grains, and number of sterile grains of barley cultivar BRS Cauê evaluated as a function of application of 2,4-D amine and choline salts at different times of crop development: before tillering, at tillering, at the first node, and after the first node. UFFS/Erechim/RS/Brazil, 2019.

| Treatments | Periods of application | | | |
|---|---|---|---|---|
| | Before tillering | At tillering | At the first node | After the first node |
| | Number of ears ($m^{-2}$) | | | |
| Without herbicide | 586.1 aA[1] | 586.1 bA | 586.1 bA | 586.1 aA |
| 2,4-D amine | 518.5 bB | 640.0 aA | 552.0 bB | 617.3 aA |
| 2,4-D choline | 457.7 cC | 556.0 bB | 643.0 aA | 470.0 bC |
| Overall average | 565.6 | | | |
| CV (%) | 5.3 | | | |
| | Length of ears (cm) | | | |
| Without herbicide | 6.5 aA[1] | 6.5 aA | 6.5 abA | 6.5 aA |
| 2,4-D amine | 6.5 aA | 6.4 aAB | 6.1 bAB | 6.0 bB |
| 2,4-D choline | 6.6 aA | 6.4 aA | 6.8 aA | 6.6 aA |
| Overall average | 6.4 | | | |
| CV (%) | 4.1 | | | |
| | Number of full grains | | | |
| Without herbicide | 18.1 bA[1] | 18.1 aA | 18.1 bA | 18.1 aA |
| 2,4-D amine | 23.4 aA | 17.1 aB | 18.5 bB | 17.2 abB |
| 2,4-D choline | 17.9 bBC | 18.5 aB | 21.9 aA | 15.9 bC |
| Overall average | 18.5 | | | |
| CV (%) | 6.2 | | | |
| | Number of sterile grains | | | |
| Without herbicide | 4.1 bA[1] | 4.1 aA | 4.1 aA | 4.1 bA |
| 2,4-D amine | 2.7 cB | 4.4 aA | 4.1 aA | 4.0 bA |
| 2,4-D choline | 6.1 aA | 4.1 aB | 3.5 aB | 5.9 aA |
| Overall average | 4.3 | | | |
| CV (%) | 12.5 | | | |

[1] Averages followed by lower case letters in the column and upper case letters in the row, do not differ by Tukey test ($p \geq 0.05$).

The greater number of full grains was inversely proportional to the number of sterile grains, with 2,4-D amine showing lower number of sterile grains for barley when applied before tillering, and 2,4-D choline at tillering and at the first node. However, the number of full grains and sterile grains can vary with the genotype or cultivar used, as well as with the herbicide applied, as denoted in a study by Galon et al. (2014).

The 2,4-D amine caused a single reduction in the hectoliter weight of barley grains when applied at tillering stage compared to its use before tillering (Table 4). And lower hectolithic weight occurred after the 2,4-D choline application at the stage after the first node, relative to the witness and the 2,4-D amine application. In the other treatments and stages of barley plant development, there were no changes in the hectoliter weight of the grains. Karpinski et al. (2018) observed that 2,4-D amine application did not cause a reduction in hectoliter weight of the barley cultivar BRS Brau.

It was observed that 2,4-D amine showed higher thousand-grain weight, when compared with 2,4-D choline and the witness without herbicide, when applied before barley tillering (Table 4). However, this value was lower than that observed with the 2,4-D choline treatment at tillering stage. 2,4-D choline showed higher thousand-grain weight when used at crop tillering. At the other stages of barley development there were no significant differences between treatments for thousand-grain weight. In a previous study, an average increment of 3% was observed in the thousand-grain weight of barley cultivars MN610 and Crioula treated with the herbicide 2,4-D amine, compared to the witness without application of the product (Galon et al., 2014). Viecelli et al. (2019) found no change in the thousand-grain weight of wheat when treated with 2,4-D amine at the third tiller emission stage.

The control without herbicide application showed higher grain yields compared to the use of 2,4-D salts at almost all stages of barley development, except at the first node stage of the crop where the treatment with 2,4-D choline showed better results (Table 4). The 2,4-D salts demonstrated higher grain yields only when applied, at tillering for the amine formulation, and at the first node stage of barley plants for the choline formulation. When comparing the application between crop development stages for each treatment, it was found that the tillering stage and after the first node for 2,4-D amine and the first node stage for 2,4-D choline demonstrate the highest barley grain yields. The 2,4-D amine was reported to not affect grain yields of wheat (Piasecki et al., 2017) and barley (Karpinski et al., 2018), which corroborates in parts with what was observed in this work.

Analyzing the data together it was possible to see that in addition to the higher grain yield, overall, 2,4-D amine applied

**Table 4.** Hectolithic weight, thousand-grain weight, and grain yield of barley cultivar BRS Cauê evaluated as a function of 2,4-D amine and choline salts application at different crop development times: before tillering, at tillering, at the first node, and after the first node. UFFS/Erechim/RS/Brazil, 2019.

| Treatments | Periods of application | | | |
|---|---|---|---|---|
| | Before tillering | At tillering | At the first node | After the first node |
| | Hectolithic weight (kg $hl^{-1}$) | | | |
| Without herbicide | 53.8 aA[1] | 53.8 aA | 53.8 aA | 53.8 abA |
| 2,4-D amine | 56.1 aA | 51.9 aB | 53.9 aAB | 55.1 aA |
| 2,4-D choline | 55.5 aA | 53.9 aAB | 56.7 aA | 51.6 bB |
| Overall average | 54.2 | | | |
| CV (%) | 3.2 | | | |
| | Thousand-grain weight (g) | | | |
| Without herbicide | 41.8 bA[1] | 41.8 bA | 41.8 aA | 41.8 aA |
| 2,4-D amine | 43.2 aA | 41.9 bB | 42.2 aAB | 42.2 aAB |
| 2,4-D choline | 41.4 bB | 43.5 aA | 41.5 aB | 41.3 aB |
| Overall average | 42.0 | | | |
| CV (%) | 1.5 | | | |
| | Grain yield (kg $ha^{-1}$) | | | |
| Without herbicide | 2,271.6 aA | 2,271.6 aA | 2,271.6 bA | 2,271.6 aA |
| 2,4-D amine | 1,793.1 bB | 2,134.4 aA | 1,779.7 cB | 1,987.3 bA |
| 2,4-D choline | 1,790.5 bBC | 1,878.6 bB | 2,713.6 aA | 1,665.7 cC |
| Overall average | 2,069.1 | | | |
| CV (%) | 4.7 | | | |

[1] Averages followed by lower case letters in the column and upper case letters in the row, do not differ by Tukey test ($p \geq 0.05$).

during tillering and 2,4-D choline used at the first-node stage showed the best responses for barley yield components such as; the number of ears, ear length, number of full grains, hectoliter weight, and thousand-grain weight (Table 4). Robinson et al. (2015) stated that the injury caused by 2,4-D amine is transient and therefore did not drastically affect the grain yield of the wheat crop.

Plant tolerance to the herbicide 2,4-D is related to limited translocation via phloem mediated by auxin receptors (T1R1/AFB), which govern biochemical and transcriptional responses (Piasecki et al., 2017). In plants, the T1R1 protein is involved in herbicide perception and substrate recognition, and it acts in the regulation of ethylene and abscisic acid synthesis. Furthermore, Mithila et al. (2011) reported that tolerant plants show rapid hydroxylation of the molecule by P450 and conjugation with amino acids, making them insensitive to auxin herbicides, thus justifying the low phytotoxicity values and maintenance of values for yield components found in the present study.

Also, the amine formulations are more water soluble, requiring a longer time to cross the leaf cuticle. Because of this, these formulations are susceptible to drift after application, and are also susceptible to precipitate formation if applied with water with high cation contents (Kumar & Singh, 2010; Sosnoskie et al., 2015). Despite improvements with respect to volatility, there are still questions about the environmental risk of this formulation, due to the extensive and often inappropriate use of the active ingredient (Marcinkowska et al., 2017).

Given this, the development of new technologies, such as the formulation with choline salt is important, because it is a quaternary ammonium salt that has as a differential the reduction of the percentage of finer droplets, volatility and odor, when compared to other formulations (Sosnoskie et al., 2015). However, studies related to the efficacy of the applications of these products in different stages of crop development is crucial to ensure the productivity and profitability of producers.

## Conclusions

The two salts of 2,4-D (choline and amine) show low phytotoxicity to the barley cultivar BRS Cauê.

Later applications (at the first node and after the first node) of the 2,4-D salts (choline and amine) are less phytotoxic than the use of the products at earlier phenological stages (before tillering and at tillering) of the barley cultivar BRS Cauê.

The tillering and first-node stages were the best for the application of 2,4-D amine and choline, respectively, with better response of grain yield components of the barley crop.

The use of 2,4-D amine at tillering stage and 2,4-D choline at the first node of barley show the highest grain yield of the crop.

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## Compliance with Ethical Standards

**Author contributions:** Conceptualization: LG, RMJ, DB, GFP; Data curation: LG, RMJ, DB, SNW, DCC, GFP; Formal analysis: LG, RMJ, DB, SNW, DCC, GFP; Funding acquisition: LG, RMJ, DB; Investigation: LG, RMJ, DB, SNW, DCC, GFP; Methodology: LG, RMJ, GFP; Project administration: LG, RMJ, GFP; Supervision: LG, DB; Validation: LG, RMJ, DB, SNW, DCC, GFP; Visualization: LG, RMJ, DB, SNW, DCC, GFP; Writing – original draft: LG, RMJ, DB, SNW, DCC, GFP; Writing – review & editing: LG, RMJ, DB, SNW, GFP.

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