

Selectivity of atrazine + mesotrione for canola as a function of application timings

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Abstract: Background: Currently, there are few herbicides registered for the control of weed species infesting canola crops. **Objective:** to evaluate the selectivity, the effects on gas exchange, and the yield components of canola when applying atrazine + mesotrione at different timings and rates before sowing, and clethodim after crop emergence. **Methods:** Phytotoxicity, gas exchange, and grain yield components of canola were evaluated after herbicide application. Field experiments were conducted over two years using a randomized block design in a 2×6+ 1+1 factorial, with four replications. The treatments were: a hand-weeded control, rates of atrazine + mesotrione (250 + 25; 500 + 50; 750 + 75 g a.i ha⁻¹) applied alone or in combination with clethodim (108 g a.i ha⁻¹). The atrazine +

mesotrione mixture was applied at 10 and 5 days before sowing (DBS), and clethodim was applied post-emergence when canola plants had three to four leaves. **Results:** The mixture atrazine + mesotrione, when applied at the highest rate, both alone and in combination with clethodim, caused high phytotoxicity to the Nuola 300 canola hybrid. Atrazine + mesotrione applied at 500 + 50 and 750 + 75 g a.i ha⁻¹, 10 days before sowing, had a negative effect on the crop's physiological variables. **Conclusions:** Atrazine + mesotrione is a promising herbicide mix that could add options for protecting yield in canola. This mixture, when applied at the rate of 250 + 25 g a.i ha⁻¹, either alone or in combination with clethodim, caused no injuries.

Keywords: *Brassica napus* L. var. *oleifera*; herbicide mixtures; injuries

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1. Introduction

Canola (*Brassica napus* L. var. *oleifera*), a member of the Brassicaceae family, originated from the genetic improvement of rapeseed through the reduction of erucic acid and glucosinolate content. This adaptation enhanced the crop's palatability (Estevez et al., 2014). It stands as the third most relevant oilseed globally in terms of grain production volume, surpassed only by soybean and oil palm. Its versatility extends to uses as green forage, protein meal for animal feed, and raw material for oil extraction with a dual purpose: human consumption and biodiesel production (Brandler et al., 2021; Costa et al., 2025).

In recent years, canola has emerged as a strategic option for crop rotation, replacing wheat and other winter cereals. This advancement is largely due to the development of management technologies that have boosted crop productivity and stability (Costa et al., 2025). Furthermore, canola adoption mitigates phytosanitary issues in grasses and legumes, thereby improving rotation systems and fostering economically viable and environmentally sustainable agricultural practices (Nichelati et al., 2020; Franz et al., 2020; Costa et al., 2025).

Although relatively new to Brazil, canola cultivation already spans 202.2 thousand hectares, with an average yield of 1,459 kg ha⁻¹ and an estimated production of 295.1 thousand tons, with Rio Grande do Sul being the leading producer (Companhia Nacional de Abastecimento, 2026). However, the average productivity remains below the potential demonstrated in experimental areas or high-tech commercial fields. This deficit primarily stems from competition with weeds of the same family (Brassicaceae), such as wild radish (*Raphanus raphanistrum* and *R. sativus*) (Franz et al., 2020; Nichelati et al., 2020; Brandler et al., 2021).

Beyond resource competition, a significant concern is the contamination of canola grains by wild radish seeds (*Raphanus* spp.). Their morphological similarity compromises the quality of the produced oil. While canola grains typically contain less than 1% erucic acid, wild radish seeds have levels approaching 15% (Franz et al., 2020). This distinction is critical because low erucic acid levels enhance the palatability of by-products, whereas high concentrations are associated with potential human toxicity (Santos et al., 2018; Ramachandran, Godara, 2025).

Another relevant weed is ryegrass (*Lolium multiflorum*), whose interference causes significant losses in grain yield and quality (Brandler et al., 2021). For its control in non-resistant biotypes, graminicide herbicides selective to canola are employed. This selectivity stems from the crop's enzymatic insensitivity to ACCase (acetyl-CoA carboxylase) inhibitors, which is the mode of action exploited by these herbicides (Vargas et al., 2011).

Weed competition can reduce canola productivity by 70% to 90%. This significant yield loss is attributed to competition for water, light, and nutrients, allelopathic effects, and the role of weeds as hosts for pests and diseases (Nichelati et al., 2020; Costa et al., 2025). Infesting species, due to their hardiness and adaptability to adverse conditions, become aggressive competitors.

In this context, the use of improved hybrids provides canola with a greater competitive advantage against weeds compared to conventional varieties. This is a crucial factor for mitigating losses and ensuring productive stability under environmental stresses (Nichelati et al., 2020; Brandler et al., 2021).

In Brazil, the registration of herbicides for weed control in canola remains limited (Costa et al., 2025), especially for post-emergence control of wild radish (*Raphanus* spp.) (Ministério da Agricultura e Pecuária, 2026). While Clearfield® technology hybrids have recently enabled the use of ALS inhibitors (such as imazamox), ensuring crop selectivity (Kozar and Domblides, 2024), conventional hybrids still lack effective chemical alternatives.

In this context, the mixture of atrazine + mesotrione emerges as a promising option. Its relevance stems from its broad spectrum of action (controlling both mono- and dicotyledonous weeds), prolonged residual activity, complementary modes of action (Photosystem II and carotenoid inhibition), and potential to mitigate herbicide resistance selection in weeds (Correia et al., 2021a; Ministério da Agricultura e Pecuária, 2026).

The herbicide mesotrione inhibits carotenoid biosynthesis by interfering with the activity of the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme in chloroplasts (Rodrigues, Almeida, 2018). In contrast, atrazine acts as a Photosystem II inhibitor, translocating via the xylem to the leaves after application. Phytotoxic symptoms are characterized by photobleaching (mesotrione) and generalized chlorosis (atrazine), ultimately progressing to necrosis and tissue death ((Rodrigues, Almeida, 2018). Complementarily, clethodim, a systemic graminicide herbicide, acts as an acetyl-CoA carboxylase (ACCase) inhibitor, blocking lipid synthesis. Its effects manifest as progressive leaf yellowing, necrosis, and destruction of the apical meristem in grass plants (Rodrigues, Almeida, 2018).

The lack of herbicide selectivity in certain crops can lead to injuries, physiological imbalance (including compromised nutrient uptake), phytotoxicity symptoms, and deregulation of plant defense mechanisms (Gaines et al.,

2020; Correia, Carvalho, 2021a; Mucheri et al., 2024). These negative effects reduce grain size, weight, and quality, as well as the number of siliques – factors directly associated with decreased canola productivity (Vargas et al., 2011; Nichelati et al., 2020).

The physiological parameters of canola can be significantly affected by herbicide use, with reductions in internal CO₂ concentration (C_i), stomatal conductance to water vapor (G_s), photosynthetic rate (A), water use efficiency (WUE), and carboxylation efficiency (CE), indicating impairment to carbon assimilation and photosynthetic efficiency. Some studies have shown that herbicides can compromise photosynthetic function and increase oxidative stress in susceptible plants, directly affecting physiological efficiency through reduced CO₂ absorption, which can impair energy production, growth, and plant productivity (Carvalho et al., 2009; Agostinotto et al., 2016; Durigon et al., 2018; Costa et al., 2025). These effects suggest that the interaction between herbicides and plants can cause a combined physiological stress that compromises the ability of canola to utilize its resources efficiently, with direct effects on grain yield.

Given the limited number of registered herbicides for weed control in canola (Costa et al., 2025; Ministério da Agricultura e Pecuária, 2026), it is essential to test new products for their selectivity. This approach aims to rotate modes of action and active ingredients, thereby reducing the risk of selecting resistant biotypes in weed species. Some herbicides are registered for use in canola, such as atrazine, clethodim, dicamba, diquat, fluazifop-P-butyl, glyphosate, imazamox, imazapyr, and S-metolachlor, which can be applied before or after sowing, or even for desiccation in seed and grain production (Ministério da Agricultura e Pecuária, 2026).

The research hypothesis is that the application of the herbicides atrazine + mesotrione prior to canola sowing and clethodim after crop emergence demonstrates selectivity, does not alter gas exchange in the plants, and does not negatively affect yield and yield components. Therefore, the objective of this study was to evaluate the phytotoxicity, the effects on gas exchange, and the grain yield components of canola when applying the commercial mixture of atrazine + mesotrione at different timings and rates before sowing with and without clethodim after crop emergence, during two growing seasons.

2. Material and Methods

2.1 Location and edaphoclimatic characteristics of the experimental area

Two field experiments were conducted at the Universidade Federal da Fronteira Sul (Federal University of Southern Frontier, UFFS), at the campus located in Erechim, Rio Grande do Sul, Brazil (27°47'30"S, 52°17'40"W, 650 m altitude), during the 2022 and 2023 growing seasons.

The region's climate is classified as Cfa (Köppen-Geiger), characterized as a humid subtropical climate with hot summers. It receives uniformly distributed precipitation, ranging from 1,100 to 2,000 mm annually, and the average temperature in the warmest month is below 22 °C. Additionally, the area experiences severe and frequent frosts, with an average occurrence of 10 to 25 days per year (Peel et al., 2007).

The soil at the experimental site is classified as a Typic Hapludox (Red Latosol) (Streck et al., 2018). Soil pH correction and fertilization were performed according to physical and chemical analysis and technical recommendations for canola cultivation (Comissão de Química e Fertilidade do Solo, 2016). The chemical and physical characteristics of the soil were: pH in water 4,8; OM = 3,3%; P = 6,3 mg dm⁻³; K = 106,0 mg dm⁻³; Al³⁺ = 0,9 cmol_c dm⁻³; Ca²⁺ = 5,1 cmol_c dm⁻³; Mg²⁺ = 3,3 cmol_c dm⁻³; effective CEC = 9,8 cmol_c dm⁻³; pH 7 CEC = 17,6 cmol_c dm⁻³; H+Al = 8,7 cmol_c dm⁻³; base saturation = 51%, clay = 62%, sand = 14 and silt = 24%.

Meteorological conditions, such as relative air humidity (%), precipitation (mm) and temperature (°C), recorded during the period of experiments can be observed in Figure 1.

2.2 Experimental design and treatments used

The study employed a randomized complete block design in a 2 × 6 factorial, supplemented with two control treatments with four replications. Factor A comprised the application timings of atrazine + mesotrione: 10 and 5 days before sowing (DBS). Factor B included six herbicide treatments (rates of the mixture applied alone or combined with clethodim), in addition to two controls: a weeded

control and clethodim applied alone (detailed in Table 1). Each plot measured 3 m × 5 m, consisting of six sowing rows spaced 0.5 m apart. For evaluations, the four central rows were considered the usable area, excluding one lateral row as a border and 1 m from each plot's ends.

2.3 Canola sowing and herbicide application

The experiments were sown using a no-till seeder-fertilizer drill on June 28, 2022, and June 27, 2023, corresponding to the first and second growing seasons, respectively. In both years, the canola hybrid 'Nuola 300' was used, which is characterized by a medium growth cycle (130–150 days) and polygenic resistance to blackleg. Seedling emergence occurred eight days after sowing. Seeding density was set at 60 viable seeds m⁻², totaling 600,000 seeds ha⁻¹, with a sowing depth of 2 cm. For base fertilization, 335 kg ha⁻¹ of 05-30-15 (N-P₂O₅-K₂O) formula was applied at sowing. Topdressing fertilization was done with 44 kg ha⁻¹ of nitrogen, equivalent to 98 kg ha⁻¹ of urea (45% N), split into two applications: the first, 49 kg ha⁻¹ of urea, at the two to four-leaf stage, and the second, with the same rate, at the six to eight-leaf stage, in both growing seasons.

Herbicide applications were carried out using a precision CO₂-pressurized backpack sprayer equipped with four DG 110.02 flat-fan spray tips, a constant pressure of 210 kPa and a travel speed of 3.6 km h⁻¹, which resulted in a spray volume of 150 L ha⁻¹. Meteorological conditions during applications are available in Table 2.

In both growing seasons, atrazine + mesotrione were applied pre-emergence, either at 10 or 5 days before canola sowing. Clethodim + mineral oil, conversely, were applied post-emergence, when the crop was at the three to

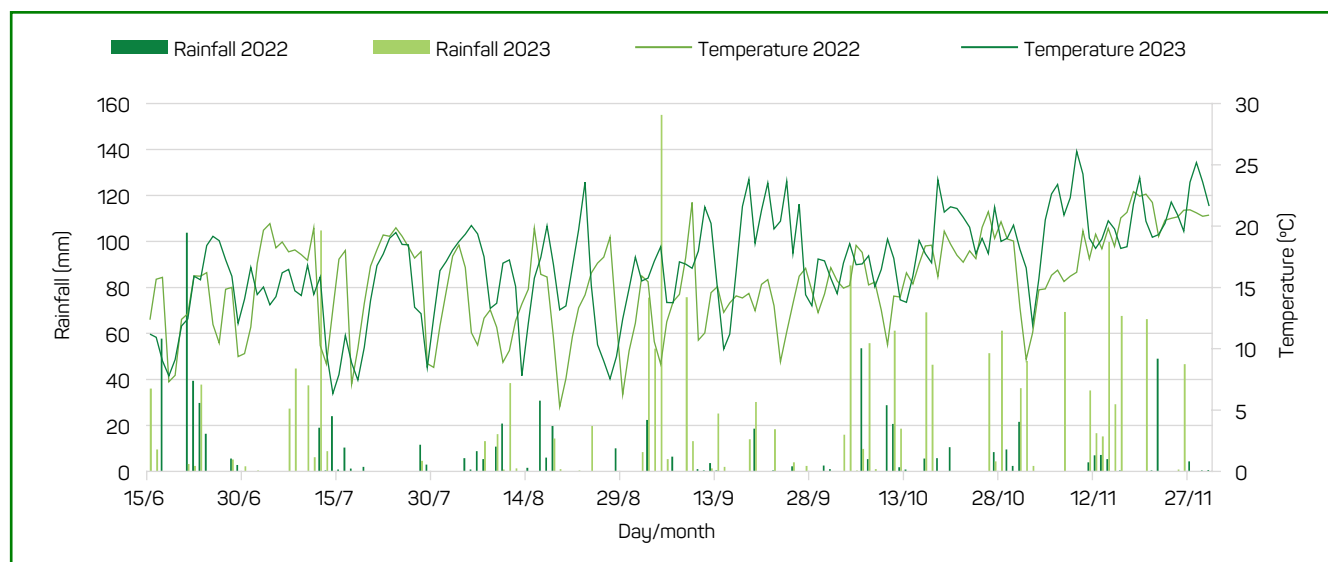


Figure 1 - Relative humidity (%), precipitation (mm), and average temperature (°C) during the experimental conduction period in the years 2022 and 2023

Table 1 - Treatments used in the experiments, their rates, commercial product, adjuvant product and application seasons (DBS - days before sowing and DAE - days after emergency) in the years 2022 and 2023. UFFS, *Campus Erechim/RS*

Treatments	Rate (g ha ⁻¹)	Commercial product	Rate (L or kg ha ⁻¹)	Herbicide application time (days)
Weeded control	-----	-----	-----	-----
Atrazine+mesotrione	250+25	Calaris	0.50	10 DBS
Atrazine+mesotrione	500+50	Calaris	1.00	10 DBS
Atrazine+mesotrione	750+75	Calaris	1.50	10 DBS
Atrazine+mesotrione	250+25	Calaris	0.50	05 DBS
Atrazine+mesotrione	500+50	Calaris	1.00	05 DBS
Atrazine+mesotrione	750+75	Calaris	1.50	05 DBS
Clethodim	108	Poquer	0.45*	30 DAE
Atrazine+mesotrione+clethodim	250+25/108	Calaris+Poquer®	0.50/0.45*	10 DBS and 30 DAE
Atrazine+mesotrione+clethodim	500+50/108	Calaris+Poquer®	1.00/0.45*	10 DBS and 30 DAE
Atrazine+mesotrione+clethodim	750+75/108	Calaris+Poquer®	1.50/0.45*	10 DBS and 30 DAE
Atrazine+mesotrione+clethodim	250+25/108	Calaris+Poquer®	0.50/0.45*	05 DBS and 30 DAE
Atrazine+mesotrione+clethodim	500+50/108	Calaris+Poquer®	1.00/0.45*	05 DBS and 30 DAE
Atrazine+mesotrione+clethodim	750+75/108	Calaris+Poquer®	1.50/0.45*	05 DBS and 30 DAE

* Adjuvant Assist added to the rate of 0.5% v/v.

Table 2 - Environmental conditions at the time of application of herbicides in pre and post-emergence of the 'Nuola 300' canola hybrid. UFFS, *Erechim Campus/RS*

Year	Application		Sky	Temp. (°C)		UR (%)	Soil conditions	Wind speed (km h ⁻¹)	Sowing	Emergence
	Time	Date		air	soil					
Pre-emergence application of atrazine + mesotrione										
2022	10 DBS	15 jun	Clear	21.5	12	46.5	Friable	0-3	28 jun	07 jul
2022	5 DBS	20 jun	Clear	15.5	10	79.5	Dry	4		
2023	10 DBS	17 jun	Cloudy	14.0	14	57.6	Moist	4-6	27 jun	05 jul
2023	5 DBS	22 jun	Cloudy	21.0	19	74.0	Moist	3		
Post-emergence application of clethodim										
2022	23 DAE	30 jun	Clear	20.0	15	69	Friable	4	28 jun	07 jul
2023	27 DAE	01 ago	Clear	23.5	17	65	Friable	3	27 jun	05 jul

DBS: days before canola sowing; DAE: days after canola emergence.

four-leaf stage. Any weed that germinated and emerged in the experimental area was controlled by manual hoeing whenever necessary to prevent competition with canola.

2.4 Variables evaluated

Phytotoxicity of the herbicides on the 'Nuola 300' canola hybrid was visually assessed at 7, 14, 21, 28, 35, and 42 days after clethodim application (DAA), corresponding to 30, 37, 44, 51, 58, and 65 days after crop emergence in the first growing season, and 34, 41, 48, 55, 62, and 69 days after emergence in the second season. The visual

estimation of phytotoxicity was expressed as a percentage, where 0% indicated no symptoms and 100% represented complete plant death, following the methodology described by Velini et al. (1995).

Gas exchange evaluations were performed at 45 days after emergence (DAE) of canola using an infrared gas analyzer (IRGA, model LCpro-SD, ADC BioScientific Ltd, UK). The measured variables were: internal CO₂ concentration (C_i; μmol mol⁻¹); transpiration rate (E; mol m⁻² s⁻¹); stomatal conductance (G_s; mol m⁻¹ s⁻¹); photosynthetic rate (A; μmol m⁻² s⁻¹); water use efficiency (WUE = A/E; mol CO₂ mol H₂O⁻¹); and carboxylation

efficiency ($CE = A/C_i$; mol CO_2 m^{-2} s^{-1}). Measurements were conducted between 8:00 and 11:00 h, under clear sky conditions and natural illumination, ensuring environmental homogeneity across all experimental blocks during evaluations.

Before harvest, ten canola plants were randomly collected from the usable area of each plot, placed in plastic bags, and sent to the laboratory for evaluation of yield components: number of siliques per plant (NSP), number of grains per silique (NGS), and number of grains per plant (NGP). Yield was determined by manually harvesting 6 m^2 of the center area of each plot. Thousand-grain weight was determined by counting eight samples of 100 grains each sample, with an electronic seed counter (model CSP-10Seed, Celmi) and weighting them on an analytical scale. All results were standardized to 10% moisture content, with yield values extrapolated to $kg\ ha^{-1}$.

2.5 Statistical analysis

Data from the two growing seasons were subjected to preliminary analyses to verify the suitability of a combined analysis. Residual normality was assessed, and homogeneity of variances between years was tested using Bartlett's test. No significant differences between years were detected for any of the evaluated variables ($p > 0.05$). Additionally, the analysis of variance indicated no significant year \times treatment interaction and no significant main effect of year. Therefore, the data were pooled and analyzed jointly in order to increase experimental precision. After confirmation of the assumptions, the data were subjected to analysis of variance using the F test, and when significant, means were compared using Tukey's test or the t-test, both at a 5% probability level. Statistical analyses were performed using the Sisvar software, version 5.6 (Ferreira, 2011).

3. Results and Discussion

3.1 Phytotoxicity of herbicides to canola

No significant interaction was observed between herbicides and application timing for phytotoxicity evaluated at 7, 14, 21, 28, 35, and 42 DAT (Table 3).

Herbicide treatments significantly affected phytotoxicity at all evaluation times, whereas application timing had no significant effect (Table 3). The highest phytotoxicity levels were observed with atrazine + mesotrione at $750 + 75\ g\ ha^{-1}$, both applied alone and in combination with clethodim, consistently across all evaluation periods. This response reflects the limited tolerance of canola to these herbicides, likely associated with restricted capacity for detoxification and metabolism (Correia, 2021b; Mucheri et al., 2024).

Plant tolerance to herbicides depends on processes such as absorption, translocation, metabolism, and exudation (Carvalho et al., 2009; Gwatidzo et al., 2023).

Mechanisms including reduced herbicide movement, enhanced detoxification, compartmentalization, altered target-site sensitivity, and enzyme overexpression may contribute to selectivity (Gaines et al., 2020; Mucheri et al., 2024).

Treatments containing clethodim did not increase phytotoxicity compared to those without it, and in several cases showed values close to the weeded control, with symptoms disappearing by 42 DAT (Table 3). This behavior is consistent with its mode of action as a graminicide, to which dicotyledonous species such as canola are inherently less sensitive due to enzymatic insensitivity (Vargas et al., 2011).

The lowest rate of atrazine + mesotrione ($250 + 25\ g\ ha^{-1}$), applied alone or in combination with clethodim, resulted in phytotoxicity below 8% throughout the evaluation period, with values close to the control, especially at later assessments. Intermediate phytotoxicity levels were observed for the other treatments. These results indicate that canola shows greater selectivity to this mixture at lower rates, likely due to its capacity for recovery over time. Herbicide-induced phytotoxicity is influenced by crop traits, herbicide characteristics, and environmental conditions following application, which determine the extent and duration of injury (Carvalho et al., 2009).

A clear dose-response relationship was observed, with increasing atrazine + mesotrione rates leading to greater phytotoxicity, regardless of application timing. However, the association with clethodim did not intensify these effects at any rate tested.

Phytotoxicity symptoms decreased progressively from 7 to 42 DAT, indicating plant recovery over time. This pattern may be associated with tissue regeneration, metabolic detoxification, and the gradual dissipation of herbicides in the environment (Carvalho et al., 2009; Tkach, Golubev, 2022; Correia et al., 2021b).

3.2 Effect of herbicides on canola physiology

No significant interaction was observed between herbicide and application timing for stomatal conductance, transpiration rate, and WUE. In contrast, internal CO_2 concentration, photosynthetic rate, and carboxylation efficiency showed significant interaction between factors.

The physiological analyses (Table 4) revealed that the application of atrazine + mesotrione resulted in the lowest physiological performance of canola among the herbicide treatments. These changes reflect the disruptive effects that these compounds can have on the metabolic and physiological processes of plants (Correia et al., 2021b; Tkach, Golubev, 2022), a pattern that has been consistently documented in other studies with canola (Durigon et al., 2018; Umurzokov et al., 2019) and other crops such as wheat (Agostinetto et al., 2016). At 10 DBS, the isolated application of atrazine + mesotrione ($750 + 75\ g\ ha^{-1}$) resulted in the lowest internal CO_2 concentration and the highest photosynthetic rate (Table 4). In contrast, lower

Table 3 - Mean phytotoxicity (%) at 10 and 5 days before canola sowing, as a function of herbicide application in the canola hybrid 'Nuola 300', cultivated in the 2022 and 2023 growing seasons. UFFS, Erechim *Campus*, RS, Brazil

Treatments	Rate (g ha ⁻¹)	Phytotoxicity to the canola hybrid 'Nuola 300 (%)					
		7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
Weeded control	---	0.00 e ¹	0.00 e	0.00 d	0.00 e	0.00 d	0.00 d
Atrazine+mesotrione	250 + 25	6.75 d	7.58 d	5.42 c	4.92 cd	1.50 cd	1.25 cd
Atrazine+mesotrione	500 + 50	15.67 bc	16.50 bc	16.25 a	13.08 ab	10.25 b	4.83 bc
Atrazine+mesotrione	750 + 75	22.00 a	21.17 ab	18.92 a	16.83a	15.00 a	10.17 a
Clethodim	108	2.50 de	7.33 d	6.67 bc	5.50 cd	2.50 cd	0.00 d
Atrazine+mesotrione+clethodim	250+25/108	6.75 d	7.25 d	5.25 c	4.00 de	0.42 d	0.00 d
Atrazine+mesotrione+clethodim	500+50/108	13.25 c	12.83 c	10.42 b	9.10 bc	5.58 c	2.50 cd
Atrazine+mesotrione+clethodim	750+75/108	20.83 ab	22.08 a	18.75 a	16.00 a	11.50 ab	7.50 ab
General means	---	10.97	11.84	10.21	8.68	5.84	3.28
C.V. (%)	---	43.17	36.42	41.68	46.35	69.33	98.46
F _{herbicides}	---	48.32	50.91	44.14	36.89	31.88	20.82
F _{application times}	---	3.23	0.007	2.05	3.23	62.34	3.20
F _{herbicides x application times}	---	1.25	0.84	1.76	1.25	25.24	1.81
P _{herbicides}	---	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
P _{application times}	---	0.08	0.93	0.16	0.08	0.06	0.08
P _{herbicides x application times}	---	0.28	0.56	0.10	0.28	0.13	0.09

¹ Means followed by the same lowercase letters in the column do not differ by Tukey's test at $p \leq 0.05$. DAT: Days after the treatments were applied. CV: coefficient of variance; F: analysis of variance; P: probability.

photosynthetic rates were observed with the isolated application of atrazine + mesotrione at 250 + 25 g ha⁻¹ and with its combination with clethodim at 500 + 50 and 750 + 75 g ha⁻¹. The association of atrazine + mesotrione with clethodim at 750 + 75 g ha⁻¹ resulted in the highest internal CO₂ concentration, although this treatment did not differ from the weeded control.

The inverse relationship observed between internal CO₂ concentration and photosynthetic rate indicates that plants with greater photosynthetic activity were more efficient in utilizing available CO₂, resulting in lower internal concentrations of this gas. Similar responses have been reported in canola and other crops subjected to herbicide stress (Agostinetto et al., 2016; Durigon et al., 2019; Galon et al., 2023). The increased photosynthetic rate observed with the isolated application of atrazine + mesotrione at 750 + 75 g ha⁻¹ may reflect a temporary compensatory physiological response under moderate stress conditions, possibly associated with recovery processes or increased chlorophyll content, as previously suggested by Durigon et al. (2019).

Ten days after sowing, the isolated application of atrazine + mesotrione at 750 + 75 g ha⁻¹ also resulted in the highest carboxylation efficiency (CE), whereas its combination with clethodim resulted in the lowest CE values (Table 4). At 5 DBS, no differences in CE were observed among treatments. The reduction in CE under the combined treatment suggests additional phytotoxic effects compromising photosynthetic

metabolism, while the higher CE observed under isolated application may indicate an adaptive response of plants to moderate herbicide-induced stress.

Regarding stomatal conductance, the weeded control, the isolated application of atrazine + mesotrione at 500 + 50 g ha⁻¹, and clethodim alone showed the highest values, differing from the combined application of atrazine + mesotrione (500 + 50 g ha⁻¹) with clethodim, which resulted in the lowest stomatal conductance (Table 5). Similarly, the highest transpiration rate was observed with clethodim applied alone, whereas the lowest transpiration occurred under the combined application of atrazine + mesotrione (500 + 50 g ha⁻¹) with clethodim. These responses indicate greater stomatal restriction under the combined treatment, likely associated with increased physiological stress and partial stomatal closure to reduce water loss.

WUE, the combined application of atrazine + mesotrione (500 + 50 g ha⁻¹) with clethodim resulted in the highest values, followed by the isolated application of atrazine + mesotrione at the same rate (Table 5). Lower WUE values were observed for the isolated application of atrazine + mesotrione at 250 + 25 g ha⁻¹ and for clethodim alone. The remaining treatments showed intermediate responses. These findings suggest that the intermediate herbicide rate, particularly when combined with clethodim, promoted more efficient water use through improved stomatal regulation and coordination between carbon assimilation and water loss.

Table 4 - Internal CO₂ concentration (C_i, μmol mol⁻¹), photosynthetic rate (A, μmol m⁻² s⁻¹) and carboxylation efficiency (EC) in Nuola 300 canola hybrid plants as a function of herbicide application at different times at 10 and 5 DBS (days before sowing) in the 2022 and 2023 agricultural years. UFFS, *Campus Erechim/RS*

Treatments	Rate (g ha ⁻¹)	CO ₂ concentration	
		10 DBS	5 DBS
Weeded control	---	298.50 Aab ¹	298.50 Aa
Atrazine+mesotrione	250+25	292.17 Abc	294.00 Aa
Atrazine+mesotrione	500+50	286.67 Abc	292.00 Aa
Atrazine+mesotrione	750+75	276.50 Bc	290.33 Aa
Clethodim	108	290.50 Abc	290.50 Aa
Atrazine+mesotrione+clethodim	250+25/108	289.17 Abc	294.17 Aa
Atrazine+mesotrione+clethodim	500+50/108	297.33 Aab	289.00 Aa
Atrazine+mesotrione+clethodim	750+75/108	314.67 Aa	296.50 Ba
General means	---	293.13	
C.V. (%)	---	3.96	
F _{herbicides}	---	5.06	
F _{application times}	---	0.003	
F _{herbicides x application times}	---	2.80	
P _{herbicides}	---	0.0001	
P _{application times}	---	0.96	
P _{herbicides x application times}	---	0.01	
Treatments	Rate (g ha ⁻¹)	Photosynthetic rate	
		10 DBS	5 DBS
Testemunha capinada	---	21.00 Aab	21.00 Aa
Atrazine+mesotrione	250+25	19.98 Ab	21.11 Aa
Atrazine+mesotrione	500+50	21.96 Aab	21.15 Aa
Atrazine+mesotrione	750+75	23.22 Aa	20.65 Ba
Clethodim	108	22.33 Aab	22.33 Aa
Atrazine+mesotrione+clethodim	250+25/108	21.02 Aab	21.35 Aa
Atrazine+mesotrione+clethodim	500+50/108	20.65 Bb	22.43 Aa
Atrazine+mesotrione+clethodim	750+75/108	20.19 Ab	20.62 Aa
General means	---	21.31	
C.V. (%)	---	7.35	
F _{herbicides}	---	2.85	
F _{application times}	---	0.02	
F _{herbicides x application times}	---	2.79	
P _{herbicides}	---	0.009	
P _{application times}	---	0.90	
P _{herbicides x application times}	---	0.01	
Treatments	Rate (g ha ⁻¹)	Carboxylation efficiency	
		10 DBS	5 DBS
Testemunha capinada	---	0.07 Abc ¹	0.07 Aa
Atrazine+mesotrione	250+25	0.07 Abc	0.07 Aa
Atrazine+mesotrione	500+50	0.08 Aab	0.07 Aa
Atrazine+mesotrione	750+75	0,09 Aa	0.07 Ba
Clethodim	108	0.08 Aab	0.08 Aa
Atrazine+mesotrione+clethodim	250+25/108	0.07 Abc	0.07 Aa
Atrazine+mesotrione+clethodim	500+50/108	0.07 Bbc	0.08 Aa
Atrazine+mesotrione+clethodim	750+75/108	0.06 Ac	0.07 Aa

Continue

Continuation

General means	---	0.07
C.V. (%)	---	8.91
F _{herbicides}	---	4.60
F _{application times}	---	0.000
F _{herbicides x application times}	---	3.74
P _{herbicides}	---	0.0002
P _{application times}	---	0.99
P _{herbicides x application times}	---	0.001

¹Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ from each other by the Tukey and T tests, respectively, at $p \leq 0.05$.

DBS: days before canola sowing; CV: coefficient of variance; F: analysis of variance; P: probability.

Table 5 - Mean stomatal conductance (GS, $\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate (E, $\text{mol m}^{-2} \text{s}^{-1}$), and water use efficiency (WUE, $\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$) at 10 and 5 days before sowing of canola, as a function of herbicide application in the canola hybrid 'Nuola 300' during the 2022 and 2023 growing seasons. UFFS, Erechim Campus, RS, Brazil

Treatments	Rate (g ha^{-1})	Physiological characteristics		
		Stomatal conductance	Transpiration rate	Water use efficiency
Weeded control	---	0.75 a ¹	2.75 bc	8.92 b
Atrazine+mesotrione	250 + 25	0.70 ab	3.06 bc	6.68 bc
Atrazine+mesotrione	500 + 50	0.76 a	2.72 bc	9.26 ab
Atrazine+mesotrione	750 + 75	0.66 ab	3.35 ab	8.56 bc
Clethodim	108	0.79 a	3.74 a	7.17 bc
Atrazine+mesotrione+clethodim	250+25/108	0.74 ab	3.11 abc	8.12 bc
Atrazine+mesotrione+clethodim	500+50/108	0.61 b	2.51 c	10.73 a
Atrazine+mesotrione+clethodim	750+75/108	0.69 ab	2.77 bc	8.84 b
General means	---	0.71	3.00	8.66
C.V. (%)	---	17.30	19.65	17.27
F _{herbicides}	---	3.81	7.39	8.41
F _{application times}	---	0.40	0.03	0.82
F _{herbicides x application times}	---	0.70	1.09	0.97
P _{herbicides}	---	0.001	0.0000	0.0000
P _{application times}	---	0.53	0.86	0.37
P _{herbicides x application times}	---	0.64	0.38	0.46

¹Means followed by the same lowercase letters in the column not differ from each other by the Tukey, at $p \leq 0.05$.

CV: coefficient of variance; F: analysis of variance; P: probability.

Physiological responses to herbicide application are strongly influenced by plant genotype, developmental stage, environmental conditions, and herbicide absorption and translocation dynamics (Correia et al., 2021a,b; Mucheri et al., 2024). In addition, herbicide-induced stress may trigger metabolic adjustments associated with reactive oxygen species production, which can contribute to acclimation responses under certain conditions (Agostinetto et al., 2016; Durigon et al., 2019).

3.3 Effect of herbicides on canola yield components

Yield components, including NSP, NGP, NGS, thousand-grain weight, and grain yield, showed significant interaction between herbicide and application timing.

For the NSP, no significant differences were observed between the treatments applied at 10 DBS (Table 6). However, at 5 DBS, the isolated application of atrazine + mesotrione ($500 + 50 \text{ g ha}^{-1}$) resulted in the lowest NSP,

Table 6 - Number of siliques per plant (NSP), number of grains per plant (NGP), and number of seeds per silique (NGS) of the canola hybrid Nuola 300 based on herbicide application, in the agricultural years of 2022 and 2023. UFFS, Campus Erechim/RS

Treatments	Rate (g ha ⁻¹)	NSP	
		10 DBS	5 DBS
Weeded control	---	132.00 Aa ¹	132.00 Aab
Atrazine+mesotrione	250+25	137.63 Aa	132.43 Aab
Atrazine+mesotrione	500+50	120.33 Aa	118.87 Ab
Atrazine+mesotrione	750+75	131.53 Aa	157.17 Aab
Clethodim	108	121.43 Aa	121.43 Ab
Atrazine+mesotrione+clethodim	250+25/108	105.63 Ba	147.60 Aab
Atrazine+mesotrione+clethodim	500+50/108	133.63 Aa	167.53 Aa
Atrazine+mesotrione+clethodim	750+75/108	146.27 Aa	158.27 Aab
General means	---	135.24	
C.V. (%)	---	21.96	
F _{herbicides}	---	2.93	
F _{application times}	---	6.47	
F _{herbicides x application times}	---	1.50	
P _{herbicides}	---	0.008	
P _{application times}	---	0.01	
P _{herbicides x application times}	---	0.04	
Treatments	Rate (g ha ⁻¹)	NGP	
		10 DBS	5 DBS
Weeded control	---	2506.40Aab	2506.40 Aa
Atrazine+mesotrione	250+25	1876.80 Bc	2289.27 Aab
Atrazine+mesotrione	500+50	1521.53 Ac	1839.70 Abc
Atrazine+mesotrione	750+75	1997.57Abc	2108.13 Aabc
Clethodim	108	1653.80Ac	1653.80 Ac
Atrazine+mesotrione+clethodim	250+25/108	1721.90Ac	1544.40 Ac
Atrazine+mesotrione+clethodim	500+50/108	1766.33Bc	2429.40 Aab
Atrazine+mesotrione+clethodim	750+75/108	2701.90Aa	1995.40 Babc
General means	---	2007.05	
C.V. (%)	---	19.51	
F _{herbicides}	---	11.23	
F _{application times}	---	1.25	
F _{herbicides x application times}	---	4.49	
P _{herbicides}	---	0.0000	
P _{application times}	---	0.27	
P _{herbicides x application times}	---	0.0002	
Treatments	Rate (g ha ⁻¹)	NGS	
		10 DBS	5 DBS
Weeded control	---	19.39 Aa	19.39 Aa
Atrazine+mesotrione	250+25	13.91 Babc	17.99 Aab

Continue

Continuation

Atrazine+mesotrione	500+50	13.24 Ac	15.73 Aabc
Atrazine+mesotrione	750+75	15.46 Aabc	13.37 Abc
Clethodim	108	13.64 Abc	13.64 Abc
Atrazine+mesotrione+clethodim	250+25/108	16.46 Aabc	11.10 Bc
Atrazine+mesotrione+clethodim	500+50/108	13.31 Ac	15.10 Aabc
Atrazine+mesotrione+clethodim	750+75/108	19.10 Aab	12.89 Bbc
General means	---		15.23
C.V. (%)	---		24.05
F _{herbicides}	---		4.32
F _{application times}	---		1.04
F _{herbicides x application times}	---		4.01
P _{herbicides}	---		0.0003
P _{application times}	---		0.31
P _{herbicides x application times}	---		0.0006

¹ Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ from each other by the Tukey and t tests, respectively, at $p \leq 0.05$. DBS: days before canola sowing; CV: coefficient of variance; F: analysis of variance; P: probability.

whereas the same treatment combined with clethodim showed the best performance for this variable.

The absence of differences at 10 DBS may be related to the longer interval between application and the reproductive stages, allowing more time for physiological recovery from herbicide-induced phytotoxic effects. In this context, the stress refers to metabolic adjustments required for herbicide detoxification rather than to weed competition, since all the plots were kept free of weeds by hand during the duration of the study. According to Carvalho et al. (2009), herbicide molecules or their metabolites may be conjugated with sugars, amino acids, or glutathione, increasing their solubility and reducing phytotoxicity. These detoxification processes require energy and metabolic resources, which may temporarily affect plant performance. On the other hand, at 5 DBS, the lower response observed for the isolated treatment may be associated with a higher metabolic cost to cope with herbicide effects, whereas the combined application with clethodim may have reduced this burden, resulting in a more favorable physiological response.

Additionally, it was observed that the combination of atrazine + mesotrione ($500 + 50 \text{ g ha}^{-1}$) with clethodim provided a higher photosynthetic rate, which may have positively contributed to the increase in the NSP.

The NGP was significantly higher at 10 DBS with the application of atrazine + mesotrione ($750 + 75 \text{ g ha}^{-1}$) combined with clethodim, being statistically similar to the weeded control (Table 6). All other treatments, whether applied alone or in combination, showed lower NGP at 10 DBS compared to the best-performing treatments (atrazine +

mesotrione at $750 + 75 \text{ g ha}^{-1}$ combined with clethodim and the weeded control). At 5 DBS, the weeded control, the isolated application of atrazine + mesotrione ($250 + 25 \text{ g ha}^{-1}$), and the combination of atrazine + mesotrione ($500 + 50 \text{ g ha}^{-1}$) with clethodim resulted in the highest NGP values, whereas the remaining treatments showed lower levels.

The increase in the number of grains with the higher rate ($750 + 75 \text{ g ha}^{-1}$) may be related to the induction of moderate stress that promoted an adaptive physiological response by the plants, favoring recovery and the increase in grain formation. On the other hand, treatments with clethodim alone or in combination with the lower rate of atrazine + mesotrione ($250 + 25 \text{ g ha}^{-1}$) probably did not impose sufficient selective pressure to stimulate such adaptive mechanisms, resulting in lower expression of reproductive potential.

Similar results were reported by Mayerová et al. (2018), who observed significant increases in crop, and among them canola yield due to the good performance of herbicide combinations. Similarly, Costa et al. (2025) identified the mixtures S-metolachlor + clomazone and quinclorac + dicamba as the most effective both in weed control and in increasing canola grain yield.

When comparing application times, it was found that the application of atrazine + mesotrione ($250 + 25 \text{ g ha}^{-1}$) alone and atrazine + mesotrione ($500 + 50 \text{ g ha}^{-1}$) associated with clethodim resulted in lower NGP when performed at 10 DBS, compared to applications at 5 DBS (Table 6). On the other hand, the treatment with atrazine + mesotrione ($750 + 75 \text{ g ha}^{-1}$) associated with clethodim showed worse performance at 5 DBS compared to the application at 10 DBS. For the other treatments,

no significant differences were observed between the application times for this variable.

These results indicate that the effect of atrazine + mesotrione rates on NGP depends on the application timing. At 5 DBS, the lower rates (250 + 25 and 500 + 50 g ha⁻¹) were associated with higher NGP values, suggesting that moderate stress may have induced adaptive responses that favored grain formation. In contrast, the highest rate (750 + 75 g ha⁻¹), particularly when combined with clethodim, resulted in lower NGP at this application timing, possibly due to insufficient time for crop recovery before the establishment of reproductive structures. However, when applied at 10 DBS, the same high-rate treatment combined with clethodim led to the highest NGP (2701.90), indicating that the longer interval between application and sowing allowed physiological recovery and even a compensatory response by the plants.

For both application times (10 and 5 DBS), the weeded control showed the highest values of NGS (Table 6). At 10 DBS, the lowest NGS values were observed with the application of atrazine + mesotrione (500 + 50 g ha⁻¹), both alone and in association with clethodim. At 5 DBS, the treatment with atrazine + mesotrione (250 + 25 g ha⁻¹) followed by the application of clethodim showed the lowest NGS, differing only from the control. These results indicate that the impact of herbicides on NGS is mainly related to the applied rate and the physiological sensitivity of the plant, as when no herbicides were applied, the crop expressed its highest reproductive potential. Comparing the application times, it was observed that the lowest NGS values occurred with atrazine + mesotrione (250 + 25 g ha⁻¹) applied at 10 DBS, as well as with the combinations 250 + 25 and 750 + 75 g ha⁻¹ associated with clethodim and applied at 5 DBS (Table 6). For the other treatments, there was no significant difference between the times. The mode of action of herbicides, whether alone or in mixture, can interfere with the development of the plant's reproductive structures, such as flowers and siliques, reducing the NGS. Moreover, interactions between active ingredients (synergism, antagonism, or additive effects) can intensify or mitigate negative physiological effects, being influenced by factors such as the plant's developmental stage, environmental conditions, soil characteristics, and crop management (Carvalho et al., 2009; Costa et al., 2025).

At 10 DBS, the rates of atrazine + mesotrione (250 + 25 and 750 + 75 g ha⁻¹) associated with clethodim showed the best and worst results, respectively, for the thousand-grain weight (TGW) (Table 7). The treatments at 5 DBS did not show a significant effect on the variable under study. It is noted that, at the lower rate (250 + 25 g ha⁻¹), the association of atrazine + mesotrione with clethodim exhibited a degree of selectivity, allowing canola plants to degrade or metabolize the herbicide mixture, thus

minimizing damage to the crop. On the other hand, when the association was applied at the higher rate (750 + 75 g ha⁻¹), the plants were unable to recover, resulting in reduced TGW when the herbicide mixture was applied at 10 DBS.

Differences in treatment application were observed, specifically for the combination of atrazine + mesotrione (500 + 50 and 750 + 75 g ha⁻¹), both associated with clethodim para a variavel TGW. The highest TGW was recorded when these treatments were applied at 5 DBS, compared to application at 10 DBS (Table 7). For the other treatments, no significant differences were identified between applications made at 10 and 5 DBS. Galon et al. (2023) reported that the Diamond canola cultivar had the highest TGW in treatments that included the weeded control, in addition to the application of pendimethalin and fluzafop-p-butyl, when investigating the effect of various herbicides on the crop. Thus, as mentioned above, it is observed that the TGW is influenced by the product used, the rate, the mode and time of herbicide application, as well as climatic and soil conditions, among other factors.

There was an interaction between herbicide rate and application timing with respect to grain yield (Table 7). The application of atrazine + mesotrione (750 + 75 g ha⁻¹) alone at 10 DBS resulted in the lowest canola grain yield among the treatments. At this herbicide rate, when applied at 10 DBS, canola plants were unable to fully overcome the toxic effects of the herbicide, resulting in reduced yield. Moreover, the application of atrazine + mesotrione (750 + 75 g ha⁻¹) resulted in the highest levels of phytotoxicity (Table 3), which helps explain the reduced grain yield observed for this treatment. In contrast, the application of atrazine + mesotrione (500 + 50 g ha⁻¹) combined with clethodim at 10 DBS resulted in the highest grain yield, surpassing the weeded control by approximately 100 kg ha⁻¹; however, it did not differ statistically from the weeded control. At 5 DBS, grain yield did not differ statistically among treatments.

The low phytotoxicity observed at 42 DAT (2.50%) suggests that canola plants were able to partially recover from herbicide-induced stress under most treatment conditions, which may have contributed to the maintenance of grain yield. Similar results were reported by Vargas et al. (2011) for graminicide herbicides such as clethodim, in which no negative effects on grain yield were observed.

When comparing application timings within each treatment, differences in grain yield were observed for atrazine + mesotrione applied alone at 250 + 25 and 750 + 75 g ha⁻¹ (Table 7). For the lower herbicide rate (250 + 25 g ha⁻¹), grain yield was reduced when the treatment was applied at 5 DBS, whereas for the higher rate (750 + 75 g ha⁻¹), the reduction occurred at 10 DBS. No differences between application timings were observed

Table 7 - Thousand grain weight (TGW - g) and grain yield (kg ha⁻¹) of the 'Nuola 300' canola hybrid based on herbicide application in the years 2022 and 2023. UFFS, Campus Erechim/RS

Treatments	Rate (g ha ⁻¹)	TGW	
		10 DBS	5 DBS
Weeded control	---	2.90 Aab ¹	2.90 Aa
Atrazine+mesotrione	250+25	2.95 Aab	2.95 Aa
Atrazine+mesotrione	500+50	2.94 Aab	3.01 Aa
Atrazine+mesotrione	750+75	2.93 Aab	2.92 Aa
Clethodim	108	2.91 Aab	2.91 Aa
Atrazine+mesotrione+clethodim	250+25/108	3.02 Aa	2.98 Aa
Atrazine+mesotrione+clethodim	500+50/108	2.91 Bab	3.04 Aa
Atrazine+mesotrione+clethodim	750+75/108	2.81 Bb	3.01 Aa
General means	---	2.94	
C.V. (%)	---	4.01	
F _{herbicides}	---	1.60	
F _{application times}	---	4.57	
F _{herbicides x application times}	---	2.14	
P _{herbicides}	---	0.14	
P _{application times}	---	0.03	
P _{herbicides x application times}	---	0.04	
Tratamentos	Rate (g ha ⁻¹)	Yield	
		10 DBS	5 DBS
Weeded control	---	1684.37 Aab	1684.61 Aa
Atrazine+mesotrione	250+25	1747.79 Aab	1401.67 Ba
Atrazine+mesotrione	500+50	1505.44Aabc	1592.96 Aa
Atrazine+mesotrione	750+75	1225.01 Bc	1699.35 Aa
Clethodim	108	1413.04 Abc	1413.04 Aa
Atrazine+mesotrione+clethodim	250+25/108	1507.02Aabc	1631.84 Aa
Atrazine+mesotrione+clethodim	500+50/108	1783.56 Aa	1684.61 Aa
Atrazine+mesotrione+clethodim	750+75/108	1504.82Aabc	1515.88 Aa
General means	---	1562.17	
C.V. (%)	---	14.25	
F _{herbicides}	---	3.68	
F _{application times}	---	0.64	
F _{herbicides x application times}	---	4.27	
P _{herbicides}	---	0.001	
P _{application times}	---	0.42	
P _{herbicides x application times}	---	0.0003	

¹ Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ from each other according to Tukey's and T tests, respectively, at p≤0.05. DBS: days before canola sowing; CV: coefficient of variance; F: analysis of variance; P: probability.

for the remaining treatments. These reductions in grain yield reached 19.80% (346 kg ha⁻¹) and 27.91% (474 kg ha⁻¹), respectively, and were associated with the higher phytotoxicity levels observed under these treatment

conditions (Table 3). The results suggest that atrazine + mesotrione applied closer to sowing, even at lower rates, or applied later at higher rates, may increase crop injury and negatively affect canola grain yield.

4. Conclusions

The application of the highest rates of the atrazine + mesotrione mixture (500 + 50 and 750 + 75 g ha⁻¹), applied alone or in association with clethodim, resulted in the highest phytotoxicity percentages to the Nuola 300 canola hybrid, regardless of application timing.

The isolated application of the lowest rate of atrazine + mesotrione (250 + 25 g ha⁻¹), as well as clethodim, showed the lowest phytotoxicity values to the crop.

Regarding the physiological variables, the application of atrazine + mesotrione at dose rates of 500 + 50 and 750 + 75 g ha⁻¹, 10 DBS, negatively affected canola physiological performance.

However, despite the high phytotoxicity and the negative effects on physiological performance observed in some treatments, the greatest yields were observed with atrazine + mesotrione (500 + 50 g ha⁻¹) combined with clethodim and with atrazine + mesotrione (250 + 25 g ha⁻¹) applied alone at 10 DBS.

Future studies are recommended to evaluate herbicide selectivity and efficacy for weed control, considering different canola hybrids and soils with distinct physicochemical characteristics, as genetic variability and soil-herbicide interactions can influence crop response and compound dynamics.

Author's contributions

All authors read and agreed to the published version of the manuscript. LG, ECG, and GLM: conceptualization

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of the manuscript and development of the methodology. ECG, DAH, and RJT: data collection and curation. GFP, and ALNG: data analysis; ECG, and IG: data interpretation. LG: funding acquisition and resources. LG, GFP, and RJT: project administration. GFP, DAH, and LG: supervision. ECG, GFP, and RJT: writing the original draft of the manuscript. LG, IG, ALNG, and ECG: writing, review and editing.

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Data Availability

The data supporting the conclusions of this study are available with the corresponding author, [Galon, L.], upon request will be provided in a basic manner and can be shared to the public.

<https://www.gov.br/conab/pt-br/atuacao/informacoes-agropecuarias/safras/safra-de-graos/>

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