

Does doses and time of 2,4-D application interfere in the physiology and wheat grains yield components?

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ABSTRACT

The identification of the application stage and correct dose of 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide is important so that wheat is not harmed. In view of this, the objective of this study was to evaluate the effect of 2,4-D doses applied at different development stages of wheat crop. The experiment was conducted in a randomized block design, arranged in a 4 × 5 factorial scheme, with four replications. In factor A, the application stages (before tillering, tillering, first node and booting) were allocated and the doses of 2,4-D (0, 349, 698, 1047 and 1396 g ha⁻¹) were allocated in factor B. The variables evaluated were phytotoxicity at 7, 14, 21, 28 and 35 days after application of the treatments (DAT), photosynthetic activity, CO₂ internal concentration, stomatal conductance, efficient water use and carboxylation efficiency. The number of spikes·m⁻², spike length and number of full and sterile grains were determined in the preharvest. Thousand grain mass, grain yield and hectoliter weight were determined after harvest. The results demonstrate that the herbicide caused phytotoxicity to wheat, being greater in increasing doses and mainly before tillering, causing grain sterility and decreased productivity. The other yield components did not present difference when increasing the dose and application in different stages as well as the physiological variables. The increase of the 2,4-D doses applied before tillering and in the booting stage caused linear decrease in wheat grain yield.

Keywords: *Triticum aestivum*; phenological stages; hormonal herbicide; winter cereal.

INTRODUCTION

Wheat crop demonstrates importance for use in human food, with a world growth of 1.2%, reaching 755 million tons in the 2020/2021 season (FAO, 2022). For the same season, the crop had an increase in the estimated area sown in Brazil, as well as in productivity and in production consequently (CONAB, 2022).

In wheat, there are many factors that end up limiting grain productivity, such as weather changes, management and especially the competition caused by weeds, which compete for nutrients, water and light (AGOSTINETTO et al., 2008; GALON et al., 2019; TAVARES et al., 2019; COLOMBO et al., 2022). When weeds infest wheat crop, they generate losses in grain productivity, quality of wheat grains, in addition to being hosts for insects and diseases. If not controlled, weeds can generate yield losses between 18% and 82% (GALON et al., 2015), in addition to increasing production costs and reducing the producer's profits (LAMENGO et al., 2013; TAVARES et al., 2019; COLOMBO et al., 2022).

In the competition with weeds for factors such as light, the plant uses a greater amount of photoassimilates for stem elongation and less for production of dry mass and leaf area, thus compromising productivity and quality of grains (AGOSTINETTO et al., 2008; LAMEGO et al., 2013). Weeds that most frequently infest wheat crop and that have caused

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greater damage due to competition or because they are problematic in relation to control are ryegrass (*Lolium multiflorum*), turnip (*Raphanus raphanistrum* and *Raphanus sativus*) and black oat (*Avena strigosa*) (LAMEGO et al., 2013). To manage these weeds, several strategies can be used, one of the most used control methods for ease, lower cost and effectiveness when compared to other methods is chemical control, with the use of herbicides (TIMOSSI; FREITAS, 2011; COLOMBO et al., 2022).

One of the herbicides widely used for weed chemical control in wheat is 2,4-dichlorophenoxyacetic acid (2,4-D), which is a synthetic auxin used in two ways, as a growth regulator and as an herbicide to control dicotyledonous weeds infesting different crops (MORTENSEN et al., 2012; SKIBA; WOLF, 2017). 2,4-D in plants generates an accumulation of abscisic acid and ethylene, which are products that induce the production of reactive oxygen species, which are responsible for increasing oxidative stress (GROSSMANN, 2010).

There are some wheat characteristics that must be considered to make correct use of the 2,4-D herbicide, such as number of tillers and phenological stages, and this product is indicated to be applied in the crop until the appearance of the first visible node (FRANCO; EVANGELISTA, 2018). Application after the first node can cause a reduction in yield components due to interference caused by the herbicide on sporogenesis (ROMAN et al., 2006).

Further studies on the use of 2,4-D can significantly help producers to apply the herbicide correctly and more consciously, minimizing damage and improving the quality of grains during the harvest.

The hypothesis of this work is that application of high doses of 2,4-D at different stages of the crop causes an increase in phytotoxicity, a negative effect on physiological variables and on the components of wheat grain yield. Therefore, the objective of this work was to study the effect on the physiological and productive characteristics of wheat when applying doses of 2,4-D at different stages of crop development.

MATERIAL AND METHODS

The experiment was carried out in the field, in the experimental area of the Federal University of Fronteira Sul (UFFS), Campus Erechim, Rio Grande do Sul state, in the 2018/19 crop year, in a no-tillage system in the straw, desiccating the vegetation with the herbicide glyphosate at a dose of 1080 g a.e.·ha⁻¹, 20 days before wheat sowing. The soil is classified as typical aluminoferric red latosoil (EMBRAPA, 2013). Soil fertility correction was carried out according to chemical analysis and following the fertilization recommendations for wheat (FRANCO; EVANGELISTA 2018).

The experiment was installed in a randomized block design, arranged in a 4 × 5 factorial scheme, with four replications. Factor A was composed by stages of application of 2,4-D herbicide (before tillering, at tillering, at the appearance of the first visible node and in boot stage) and factor B by doses of 2,4-D (0, 349, 698, 1047, and 1396 g·ha⁻¹ acid equivalent).

The experimental units had an area of 13.6 m², with 16 rows with 5 m long and spaced at 0.17 m. The wheat cultivar 'ORS Vintecincio' was sown with a seeder/fertilizer at an average density of 330 seeds·m⁻² or a density of 3,330,000 plants·ha⁻¹. As base fertilization, 350 kg·ha⁻¹ of the NPK formula 05-30-15 was used. In topdressing, 75 kg·ha⁻¹ of N in urea form was applied, in two different stages, 40% in the tillering and 60% of the dose in crop elongation.

Treatments were applied with a precision backpack sprayer, pressurized with CO₂, equipped with four DG 110.02 fan spray nozzles, under constant pressure of 2.0 kgf·cm⁻² and 3.6 km·h⁻¹ displacement speed, which provided 150 L·ha⁻¹ of herbicide spray flow. The environmental conditions at the treatments application of the treatments in the wheat crop are shown in Table 1.

Table 1. Environmental conditions at the time of treatment application on wheat. UFFS, Campus Erechim, 2018.

Application stages	Temperature (°C)	Air relative humidity (%)	Wind speed (km·h ⁻¹)	Soil temperature (°C)
Before tillering	24.4	60.0	3.6	18.1
Tillering	25.0	58.3	4.9	21.6
First node	26.0	57.0	5.1	20.0
Booting	27.3	51.0	4.8	18.8

Phytotoxicity assessments of wheat plants were carried out at 7, 14, 21, 28 and 35 days after treatment application (DAT). Percentage scores were assigned to assess phytotoxicity, with 0% corresponding to the absence of phytotoxicity to the crop and 100% for death of wheat plants (SBCPD, 1995). At 30 days after application of the last stage of crop development, variables related to the physiology of wheat plants were determined, such as photosynthetic activity (A: $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), internal concentration of CO_2 (Ci : $\mu\text{mol}\cdot\text{mol}^{-1}$), stomatal conductance (Gs: $\text{mol}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$), efficient water use (UEA: $\text{mol CO}_2\cdot\text{mol H}_2\text{O}^{-1}$) and carboxylation efficiency (EC: $\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The physiological variables of wheat were determined with an infrared gas analyzer, brand ADC, model LCA PRO (Analytical Development Co. Ltd, Hoddesdon, UK), in five plants per experimental unit, always using the last leaf completely expanded of the crop. Two blocks were measured a day, between 8:00 and 11:00 am, so that the environmental conditions were as homogeneous as possible during the analyses.

In preharvest of wheat, number of spikes- m^{-2} , number of full and sterile grains and the spike length (cm) were determined at the Laboratory for Sustainable Management of Agricultural Systems at UFFS. The number of spikes- m^{-2} was determined by counting in the center of each experimental unit, using a square measuring 0.5×0.5 m. The number of full grains and sterile grains was determined by counting and, using a graduated ruler, the length of ears was measured, after randomly harvesting 10 wheat plants in each experimental unit.

Harvest was carried out manually with a useful area of 5.1 m^2 and yield components were determined with the harvested grain mass. The hectoliter weight (HW) $\text{kg}\cdot\text{hL}^{-1}$ was determined using a Dalle Molle scale, model 40. The thousand grains mass (g) was determined by counting 8 repetitions of 100 grains each, subsequently extrapolating the mass to thousand grains. Grain yield was determined by extrapolating weights to $\text{kg}\cdot\text{ha}^{-1}$. The values of HW, thousand grain mass and grain yield were corrected for 13% moisture.

Data were submitted to variance analysis by F test, and being significant, to the quantitative factor (2,4-D doses), regressions were applied, and to the qualitative factor (wheat development stages), the average separation test Scott-Knott. All tests were performed at $p \leq 0.05$.

RESULTS AND DISCUSSION

There was interaction between the factors tested (doses and application stages) for the variables; phytotoxicity at 7, 14, 21, 28, and 35 DAT, full grains per ear and sterile grains per ear. For grain yield, there was a difference between 2,4-D doses and application stages, but there was no interaction between the factors. There was no interaction between the factors and no significant difference between their levels for the physiological variables (photosynthetic activity, internal CO_2 concentration, stomatal conductance, efficient water use, and carboxylation efficiency), spikes- m^{-2} , spike length, TW and thousand grains mass of wheat.

The results demonstrate application of herbicide 2,4-D herbicide caused phytotoxicity of 5% to 15%, at 7 DAT, being lower for low doses and higher for high doses and applications before tillering. (Table 2). At 14 DAT, there was an increase in phytotoxicity for application before the tillering stage and a decrease for applications made later. In the evaluations in 21 and 28 DAT, decreases in phytotoxicity were observed for all stages.

It was observed that in application of 2,4-D before wheat tillering, the symptoms persisted until the end of evaluations, with 5% phytotoxicity, even for the lowest dose used, different from what happened in other stages, which no longer showed damage symptoms at 35 DAT even for $1047 \text{ g}\cdot\text{ha}^{-1}$ dose (Table 2). GALON et al. (2015) when evaluating the efficacy and phytotoxicity of herbicides applied for weed management in wheat, found low phytotoxicity in Quartzo cultivar with application of 2,4-D herbicide, maximum phytotoxicity obtained was 4.5% at 7 DAT, at 21 DAT there were no more symptoms.

Applications before tillering are considered early and can cause phytotoxicity, with symptoms like ear retention in the stem being very common, remaining distorted due to the attachment of the apex to the stem by the awns (ROMAN et al., 2006). Comparing the increase in 2,4-D doses, it was observed high phytotoxicity symptoms in all development stages of wheat crop, and all evaluations were adjusted to a linear equation (Fig. 1). There was a decrease in wheat phytotoxicity at each evaluation performed, this for all doses, at 35 DAT the maximum symptoms were 5%. These symptoms are caused by the effect of auxin on the inducing signals, the hormones when perceive the auxin of herbicide, interrupt the natural signals; as a consequence, there are torsions in the leaves and other deformations (IONESCU; PENESCU, 2015).

For the physiological parameters of wheat crop, such as Gs, Ci, A, UEA, and EC, there was no difference between application stages (Table 3). It is noteworthy that the visual phytotoxicity at 28 DAT did not exceed 7%, indicating recovery

of wheat plants (Table 2). In view of this fact, it is noteworthy that the herbicide, object of the study, did not change physiological characteristics of the crop, possibly due to low phytotoxicity or even to metabolism by wheat crop to a non-toxic product. In work carried out by ALTERMAN; NEPTUNE (1977), they found that wheat can metabolize 2,4-D to a nontoxic product and, thus, not affect plant's physiology or absorption of essential resources for its growth and development.

Table 2. Phytotoxicity (%) at 7, 14, 21, 28, and 35 days after application of treatments (DAT) in 'ORS Vintecinco' wheat as a function of the application of 2,4-D herbicide doses at different stages of crop development.

Application stages	2,4-D doses (g a.e.·ha ⁻¹)					CV (%)
	0	349	698	1047	1396	
Phytotoxicity (%) 7 DAT						
Before tillering	0 ^{ns}	5 ^{ns}	5 b ¹	4 c ¹	6 c ¹	11.26
Tillering	0	5	8 a	14 a	15 a	
First node	0	5	5 b	8 b	11 b	
Booting	0	5	9 a	8 b	13 a	
Phytotoxicity (%) 14 DAT						
Before tillering	0 ^{ns}	6 a ¹	12 a ¹	12 a ¹	12 a ¹	7.67
Tillering	0	3 b	7 b	10 b	11 a	
First node	0	3 b	4 c	5 c	6 c	
Booting	0	0 c	4 c	5 c	8 b	
Phytotoxicity (%) 21 DAT						
Before tillering	0 ^{ns}	5 a ¹	9 a ¹	9 a ¹	11 a ¹	15.85
Tillering	0	0 b	5 b	8 a	9 b	
First node	0	0 b	2 c	3 c	6 c	
Booting	0	0 b	0 d	4 b	7 c	
Phytotoxicity (%) 28 DAT						
Before tillering	0 ^{ns}	3 a ¹	4 a ¹	3 b ¹	6 a ¹	13.32
Tillering	0	0 b	5 a	7 a	7 a	
First node	0	0 b	0 b	2 c	6 a	
Booting	0	0 b	0 b	0 d	5 b	
Phytotoxicity (%) 35 DAT						
Before tillering	0 ^{ns}	3 a ¹	4 a ¹	3 a ¹	5 a ¹	32.56
Tillering	0	0 b	0 b	3 a	5 a	
First node	0	0 b	0 b	2 a	5 a	
Booting	0	0 b	0 b	0 b	4 b	

¹Means followed by different letters in the column do not differ by Scott-Knott test ($p < 0.05$). ^{ns} = not significant.

Regarding comparisons between 2,4-D doses, the evaluated physiological variables did not show any alteration for the moment in which the evaluation was carried out, and there was no adjustment of the data to the tested models (Fig. 2). For photosynthesis, in addition to herbicide action, there may be other factors that cause its alteration such as; light, water stress, soil characteristics, crop management, plant characteristics, and others (MATOS et al., 2013). The carboxylation efficiency is directly related to photosynthetic activity, what occurs because of stomata, which close and open, regulating

the CO₂ entry into the plant (TAIZ et al., 2017). Based on this and considering that evaluation time was distant from the applications, plant may have recovered its metabolism, degrading the herbicide to a non-toxic product and thus not showing differences for physiological parameters, as explained above.

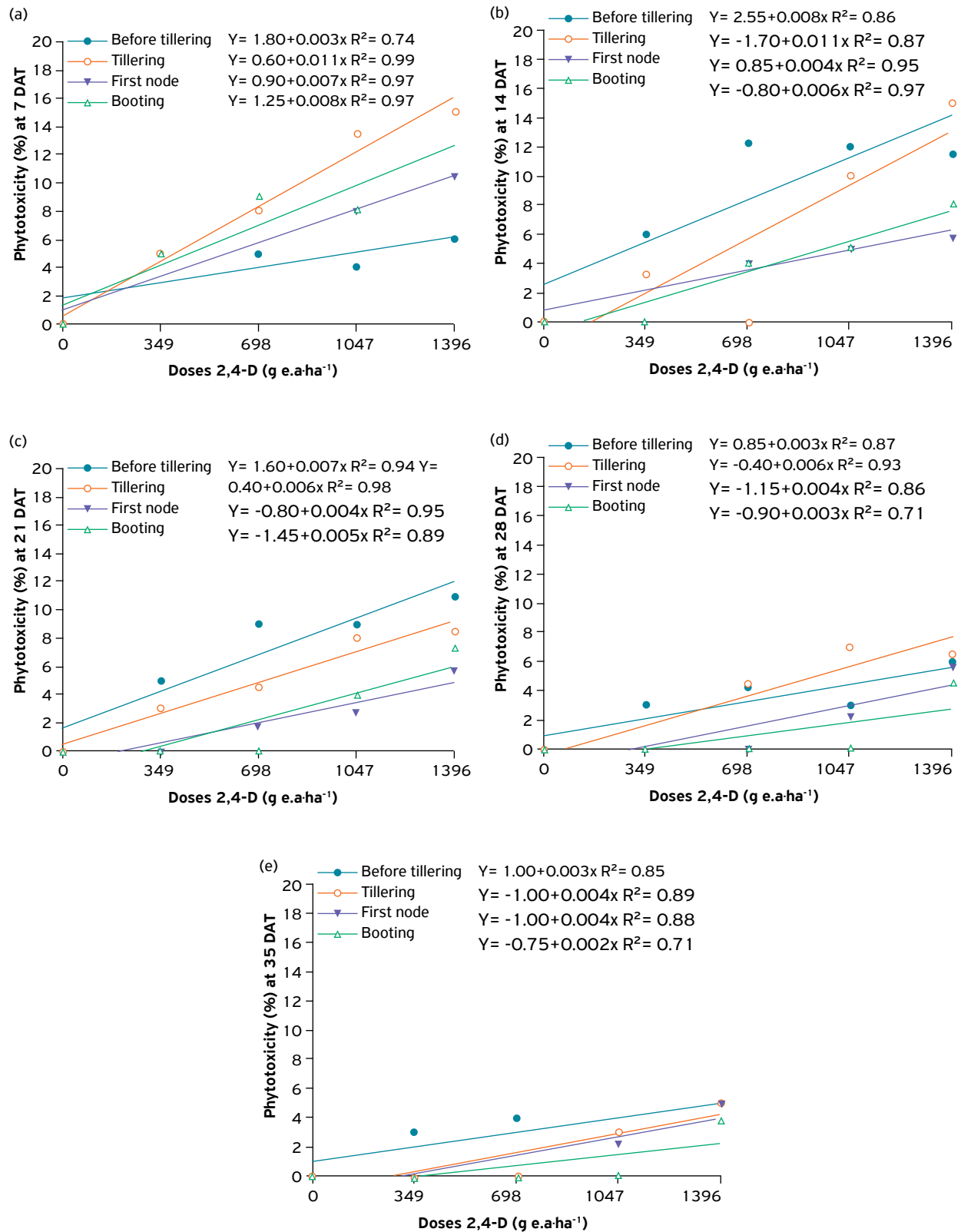


Figure 1. Phytotoxicity (%) evaluated at 7 (a), 14 (b), 21 (c), 28 (d) and 35 (e) DAT in 'ORS Vintecinco' wheat as a function of application of 2,4-D doses and development stages.

Table 3. Effect of 2,4-D herbicide applied in crop in several doses and different stages on stomatal conductance (Gs), internal CO₂ concentration (Ci), photosynthetic activity (A), efficient water use (UEA) and carboxylation efficiency (EC) of 'ORS Vintecinco' wheat.

Application stages	2,4-D doses (g a.e.·ha ⁻¹)					CV (%)
	0	349	698	1047	1396	
Stomatal conductance (mol·m⁻¹·s⁻¹)						
Before tillering	0.34 ^{ns}	0.42 ^{ns}	0.40 ^{ns}	0.37 ^{ns}	0.38 ^{ns}	16.38
Tillering	0.34	0.44	0.42	0.40	0.42	
First node	0.34	0.42	0.34	0.44	0.40	
Booting	0.34	0.38	0.41	0.41	0.40	
Internal CO₂ concentration (μmol·mol⁻¹)						
Before tillering	291.33 ^{ns}	303.42 ^{ns}	298.20 ^{ns}	300.08 ^{ns}	301.83 ^{ns}	2.83
Tillering	291.33	301.96	295.50	300.71	301.46	
First node	291.33	303.83	288.58	302.92	304.58	
Booting	291.33	391.25	300.41	302.41	291.75	
Photosynthetic activity (μmol·mol⁻¹)						
Before tillering	14.23 ^{ns}	14.12 ^{ns}	13.50 ^{ns}	12.30 ^{ns}	13.26 ^{ns}	11.59
Tillering	14.23	13.49	13.97	12.99	13.44	
First node	14.23	13.00	12.95	13.53	12.60	
Booting	14.23	13.55	13.38	13.05	14.07	
Water use efficiency (mol CO₂·mol H₂O⁻¹)						
Before tillering	3.95 ^{ns}	4.09 ^{ns}	3.95 ^{ns}	3.95 ^{ns}	4.30 ^{ns}	14.61
Tillering	3.95	3.85	3.92	3.94	4.15	
First node	3.95	3.88	4.21	4.18	4.36	
Booting	3.95	4.38	3.92	4.39	3.84	
Carboxylation efficiency (mol CO₂·m⁻²·s⁻¹)						
Before tillering	0.045 ^{ns}	0.049 ^{ns}	0.045 ^{ns}	0.041 ^{ns}	0.043 ^{ns}	13.23
Tillering	0.045	0.045	0.048	0.043	0.045	
First node	0.045	0.045	0.045	0.045	0.042	
Booting	0.045	0.047	0.045	0.044	0.049	

ns = not significant.

Regarding grain yield components, it was observed that number of spikes·m⁻², spikes length and thousand grain mass were not affected by the application of 2,4-D, as there was no significant difference between application stages, for neither herbicide doses (Table 4, Fig. 3). This fact is due to wheat plant was able to metabolize the herbicide, even when it was applied outside the recommended stage. Similar results regarding the number of spikes·m⁻² were found by RODRIGUES et al. (2006) when evaluating effect of hormonal herbicide application at different stages of wheat development.

The number of full grains per ear showed a difference for application stages in each doses evaluated, having a negative effect for the treatments where the herbicide was applied before tillering and at booting. The application before tillering generated the greatest number of sterile grains per ear, directly influencing grain yield (Table 4). Increasing 2,4-D doses linearly decreased the number of grains per ear (Fig. 4a) and increased the number of sterile grains, possible due to the increase in grain sterility (Fig. 4b). IONESCU; PENESCU (2015), when working with different herbicides in wheat, including 2,4-D, observed that number of full grains per spikes also decreases, but thousand grain mass was not

changed when applied 2,4-D isolated. RODRIGUES et al. (2006) found similar results with applications of the auxinic herbicide dicamba, resulting in a reduction in the number of full grains per spikes, which caused a loss of up to 60% in wheat grain yield.

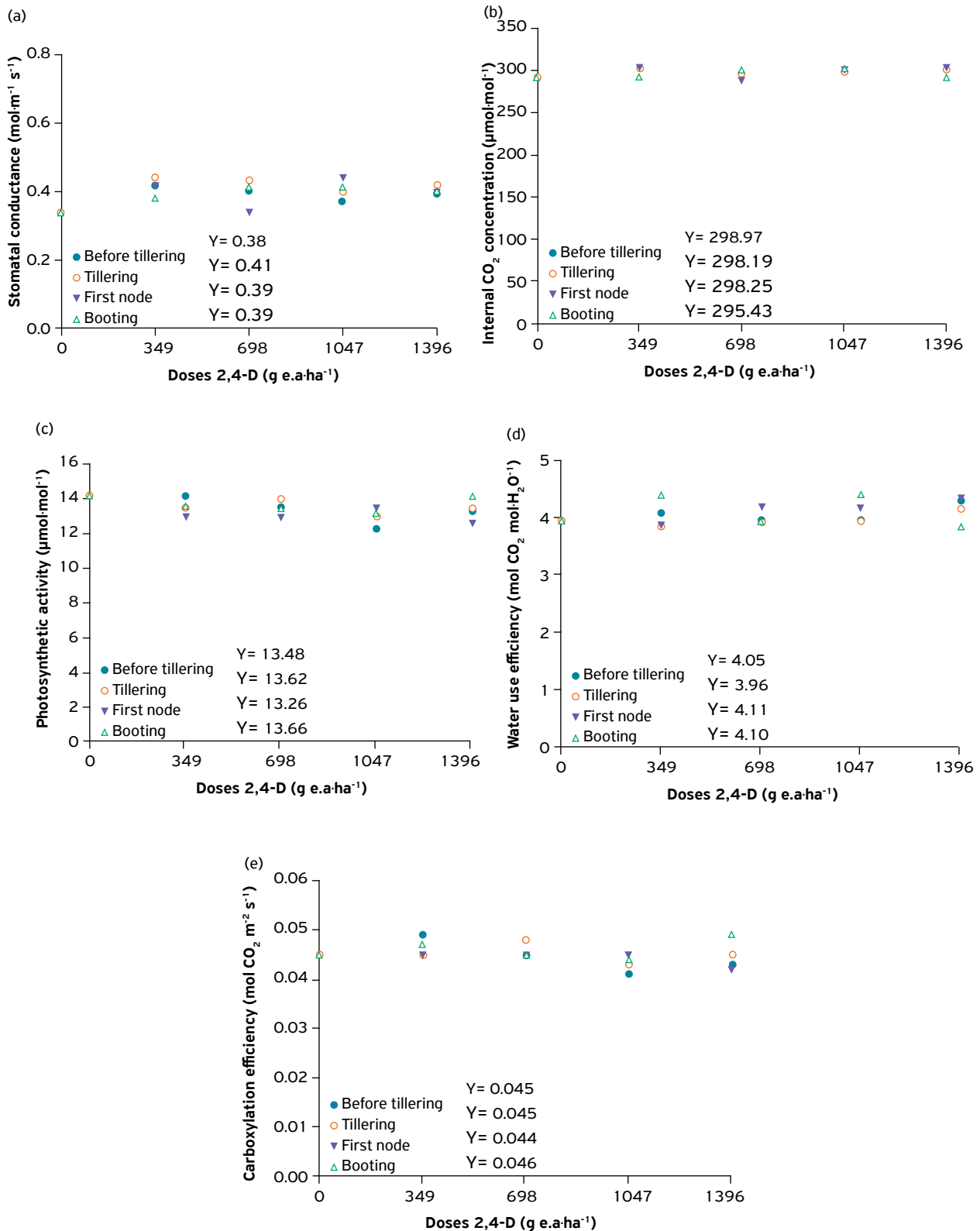


Figure 2. Effect of 2,4-D herbicide doses, applied at different crop development stages, on stomatal conductance (a), internal CO_2 concentration (b), photosynthetic activity (c), efficient water use (d) and carboxylation efficiency (e) of 'ORS Vintecinco' wheat.

Table 4. Grain·m⁻², spike length (cm), full and sterile grain·spike⁻¹, hectoliter weight (kg·hL⁻¹), thousand grain mass (g) and yield (kg·ha⁻¹) as a function of 2,4-D herbicide doses applied at several development stages in 'ORS Vintecincio' wheat.

Application stages	2,4-D doses (g a.e.·ha ⁻¹)					CV (%)
	0	349	698	1047	1396	
Spikes·m⁻²						
Before tillering	400 ^{ns}	408 ^{ns}	399 ^{ns}	399 ^{ns}	400 ^{ns}	5.19
Tillering	400	402	399	399	398	
First node	400	396	391	396	401	
Booting	400	399	402	381	388	
Length spikes (cm)						
Before tillering	9.07 ^{ns}	9.37 ^{ns}	8.97 ^{ns}	9.62 ^{ns}	9.15 ^{ns}	5.42
Tillering	9.07	9.02	8.77	9.17	9.10	
First node	9.07	9.00	8.97	9.40	8.57	
Booting	9.07	9.12	8.42	9.12	8.77	
Number of full grains·spikes⁻¹						
Before tillering	36 ^{ns}	25 c ¹	24 d	21 b	16 d	2.45
Tillering	36	29 a	29 b	28 a	23 b	
First node	36	31 a	31 a	29 a	28 a	
Booting	36	28 b	27 c	22 b	20 c	
Number of sterile grains·spikes⁻¹						
Before tillering	4 ^{ns}	7 a	8 a	14 a	13 a	6.68
Tillering	4	6 b	6 b	7 b	6 c	
First node	4	5 b	6 b	7 b	6 c	
Booting	4	6 b	6 b	13 a	12 b	
Hectoliter weight (kg·hL⁻¹)						
Before tillering	74.95 ^{ns}	74.84 ^{ns}	75.61 ^{ns}	74.87 ^{ns}	74.73 ^{ns}	1.93
Tillering	74.95	73.65	73.60	73.48	73.47	
First node	74.95	75.86	75.08	74.79	74.90	
Booting	74.95	75.70	75.55	75.08	75.45	
Thousand-grain weight (g)						
Before tillering	32.05 ^{ns}	32.42 ^{ns}	32.91 ^{ns}	32.39 ^{ns}	32.25 ^{ns}	6.68
Tillering	32.05	31.24	29.59	30.91	29.77	
First node	32.05	33.89	31.73	29.94	32.02	
Booting	32.05	30.29	31.63	29.60	31.27	
Grain yield (kg·ha⁻¹)						
Before tillering	2694.8 ^{ns}	2488.8 ^{ns}	2097.6 c	2059.7 b	2010.8 b	4.62
Tillering	2694.8	2519.6	2420.2 a	2270.8 a	2187.9 a	
First node	2694.8	2526.6	2494.1 a	2372.6 a	2171.8 a	
Booting	2694.8	2562.8	2265.3 b	2086.4 b	2033.9 b	

¹Means followed by different letters in the column do not differ by Scott-Knott test ($p < 0.05$). ^{ns} = not significant.

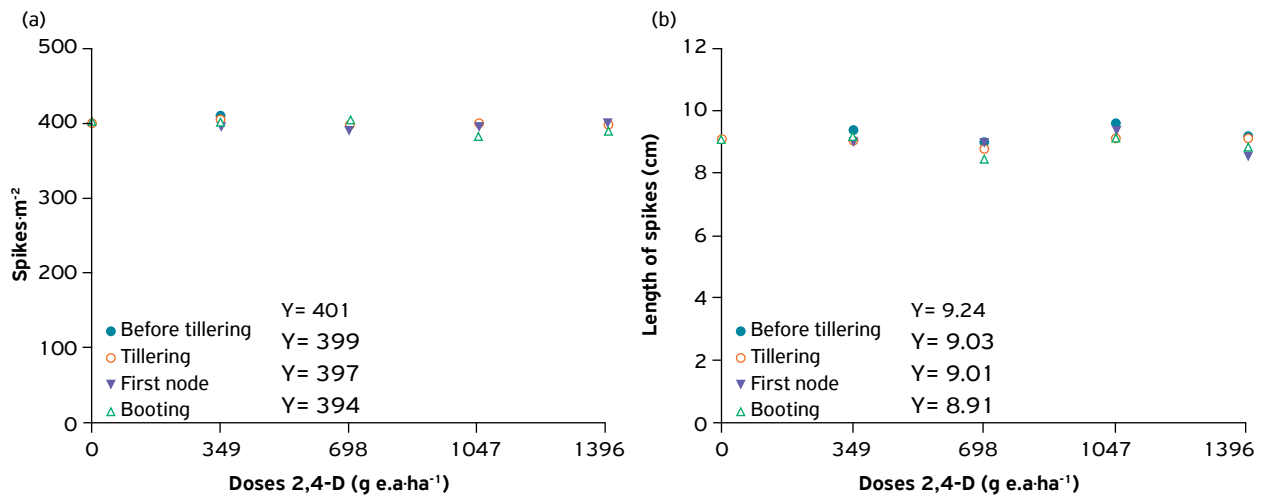


Figure 3. Effect of 2,4-D herbicide doses, applied at different crop development stages; (a) on number of spikes (m⁻²) and (b) length of spikes (cm) of 'ORS Vintecinco' wheat.

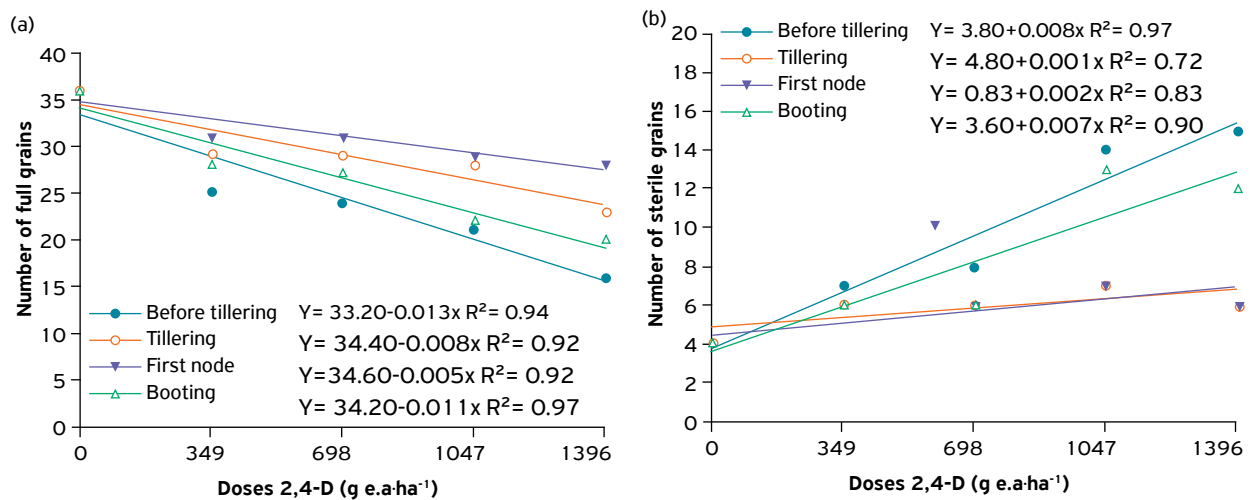


Figure 4. Effect of 2,4-D herbicide doses, applied at different crop development stages, on full (A) and sterile (B) number of grains per spikes of wheat crop.

For hectoliter weight (kg·hL⁻¹) and thousand grain mass no differences were observed between the application stages (Table 4) and no data adjustments were found for the models tested with increasing doses of 2,4-D (Fig. 5). In relation to thousand grain mass, RODRIGUES et al. (2006) found similar results when applying dicamba, having no effect on this variable.

Regarding grain yield, there was a difference between the stages in which the herbicide 2,4-D was applied, but only for doses 698, 1047 and 1396. 2,4-D use in the tillering and in the first node showed higher productivity than other stages. Early applications, such as before tillering, and later as in booting, caused decreases in wheat grain yield (Table 4). Similar results were found by TOTTMAN (1977) where early application of 2,4-D and 2-methyl-4-chlorophenoxyacetic acid affected the morphology of the plant, causing changes in leaves and ears, due to interfering in the distribution of new leaves and in the beginnings of spikelets.

With increasing doses, there was a linear decrease in grain yield for all stages in which 2,4-D was applied (Fig. 6). When comparing the application of the highest 2,4-D dose (1396 g·ha⁻¹) against the lowest (0 g·ha⁻¹), there was a reduction of 27.6, 18.8, 17.6, and 27.0% using the herbicide before tillering, at tillering, at the first node and at booting, respectively. This decrease may be linked to the effect of auxin on cell division, elongation and vascular differentiation, which in adequate doses can stimulate these processes, but in high concentrations, they affect plant growth processes and cause damage to the crop (WOODWARD; BARTEL, 2005).

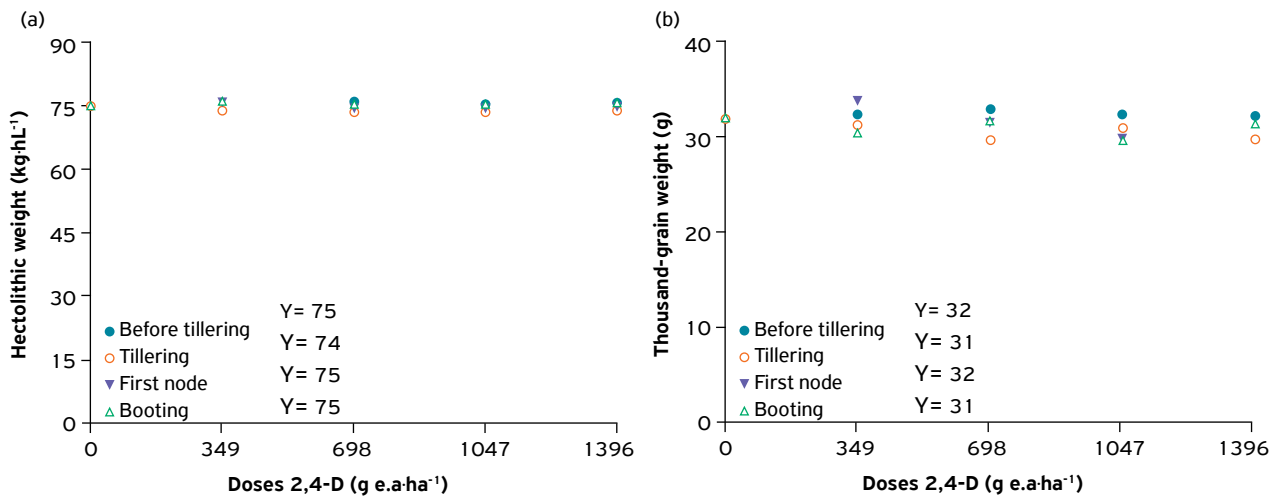


Figure 5. Effect of 2,4-D herbicide doses, applied at different crop development stages, on hectoliter weight (A) and thousand-grain weight (B) of 'ORS Vintecinco' wheat.

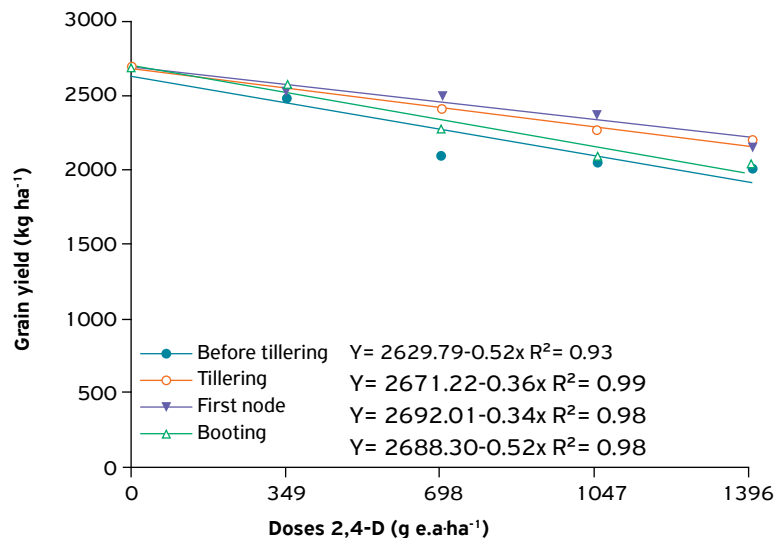


Figure 6. Effect of 2,4-D herbicide doses, applied at different crop development stages, on grain yield ($\text{kg}\cdot\text{ha}^{-1}$) of 'ORS Vintecinco' wheat.

CONCLUSIONS

The best stages to apply 2,4-D are tillering and at the first node, the application before tillering and booting resulted in grain yield losses in 'ORS Vintecinco' wheat. Neither one of the doses and application stages of 2,4-D affect the characteristics related to wheat physiology of 'ORS Vintecinco' wheat. Increasing the doses of 2,4-D herbicide, the application reduces yield of 'ORS Vintecinco' wheat, regardless the application stage.

AUTHORS' CONTRIBUTIONS

Conceptualization: Galon, L.; Soligo, V.; Forte, C.T. **Data curation:** Forte, C.T.; Perin, G.F.; Soligo, V. **Formal analysis:** Brunetto, L.; Galon, L.; Silva, A.M.L. **Funding acquisition:** Galon, L. **Investigation:** Perin, G.F.; Galon, L.; Soligo, V. **Methodology:** Soligo, V.; Forte, C.T.; Brunetto, L.; Galon, L. **Project administration:** Galon, L.; Perin, G.F. **Supervision:** Forte, C.T.; Galon, L.; Perin, G.F. **Validation:** Galon, L.; Soligo, V. **Visualization:** Galon, L.; Gallina, A.; Soligo, V. **Writing – original draft:** Soligo, V.; Galon, L.; Forte, C.T. **Writing – review & editing:** Perin, G.F.; Galon, L.; Soligo, V.; Gallina, A.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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