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Article

Physiological Performance of Seeds and Level of Herbicide Residues Depending on Application of 2.4-D in Wheat

Desempenho Fisiológico de Sementes e Nível de Resíduo em Razão da Aplicação de 2,4-D no Trigo

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ABSTRACT - Horseweed control through application of 2.4-D in the winter season is an alternative to manage biotypes resistant to herbicides that belong to other mechanisms of action. The objectives of this study were to assess the physiological quality of wheat seeds and determine the rate of 2.4-D residue, as function of application stages and herbicide rates. The treatments were arranged in factorial scheme, with three application stages (flowering, soft dough and hard dough), and four 2.4-D rates (0; 504; 1,008 and 2,015 g a.i. ha^{-1}). The application of 2.4-D in the wheat crop changed physiological seed quality by increased the rate and total percentage of germination. The application of 2.4-D resulted in herbicide accumulation in seeds, especially when application was carried out at the soft dough stage. Also, the increase in herbicide rate increased the level of residue in the seeds. However, regardless of stage of application for 2.4-D and herbicide rate in use, the values of residue found in the seeds were below the allowed maximum limit.

Keywords: *Triticum aestivum*, weeds, germination, dry matter.

RESUMO - O controle de buva através da aplicação de 2,4-D na estação hibernal de cultivo é alternativa para o manejo de biótipos com resistência a herbicidas pertencentes a outros mecanismos de ação. Os objetivos do estudo foram avaliar a qualidade fisiológica das sementes de trigo e determinar o resíduo de 2,4-D, em função do estádio de aplicação e de doses do herbicida. Os tratamentos foram arranjados em esquema fatorial, sendo três estádios de aplicação (florescimento, grão pastoso e massa firme) e quatro doses do herbicida 2,4-D (0, 504, 1.008 e 2.015 g i.a. ha^{-1}). A aplicação de 2,4-D na cultura do trigo alterou a qualidade fisiológica das sementes, resultando em aumento da velocidade e da porcentagem total de germinação. A aplicação do 2,4-D resultou em acúmulo de resíduo do herbicida nas sementes, principalmente quando a aplicação foi feita no estádio de grão pastoso, e o aumento da dose herbicida elevou a concentração de resíduo. Contudo, independentemente da época em que o 2,4-D foi aplicado, assim como da dose utilizada, os valores de resíduo observados nas sementes encontraram-se abaixo do limite máximo permitido.

Palavras-chave: *Triticum aestivum*, plantas daninhas, germinação, matéria seca.

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INTRODUCTION

Wheat (*Triticum aestivum*) is an annual species, considered the main crop in the winter growing season in the Southern region of Brazil. This cereal is extremely important in human food, as it provides energy by means of products originated from wheat processing. In the 2013/14 harvest, the cultivated area had a 10% increase compared with the previous harvest. This was due to the development of new cultivars (Trindade et al., 2006), as well as an increase in grain price.

Several factors can limit wheat yield; one of the major obstacles is weed competition (Agostinetto et al., 2008; Lamego et al., 2013). Integrated management of wheat plants is mainly performed by using preventive, cropping and chemical methods, with the latter being the most frequently used. Chemical control is based on herbicide application at pre- or post-emergence of the crop and weeds. One of the weeds that occur in wheat crops is horseweed (*Conyza* spp.) (Vargas et al., 2007).

Horseweed is an annual species, native to the Americas, and it belongs to the class Magnoliopsida and the family Asteraceae; it is predominantly autogamous (Vargas and Gazziero, 2009). This species is considered as one of the top 10 weeds that occur worldwide. It infests more than 40 crops and causes yield loss in several agricultural species (Lazaroto et al., 2008). Biotypes of this species are resistant to glyphosate, which complicates management of crops to be grown in the summer growing season. The occurrence of resistant weeds in cropping areas has led to an increase in production costs for the cultivated species. Therefore, alternative herbicides have to be used instead (Lamego et al., 2013).

Horseweed management can be performed in the winter by applying auxin mimic herbicides, such as 2.4-dichlorophenoxyacetic acid (2.4-D) (Vargas et al., 2007). It is an efficient tool in reducing the number and size of weed plants (Vargas and Gazziero, 2009). Larger horseweed plants are controlled more easily than smaller ones (Vargas and Gazziero, 2009). Weed development at the stage of application affects herbicide efficiency; in the case of horseweed, satisfactory control is achieved when the herbicide is applied up to a plant height of 10 cm (Oliveira Neto et al., 2010). However, the application of hormonal herbicides at an inadequate stage or at an inadequate rate can affect the growth and development of wheat plants, causing a reduction in grain yield (Rodrigues et al., 2006). Furthermore, in many cases, herbicide application can also affect the physiological quality of seeds (Toledo et al., 2012).

Germination, dormancy and vigor are seed attributes which define the physiological quality of these structures (Peske et al., 2012). Germination is determined by the germination test, and the results are used primarily for the purpose of marketing seed lots (Peske et al., 2012). In its turn, vigor indicates the ability of seeds to originate a normal plant in field conditions (Moterle et al., 2011). Tests such as field emergence, seedling length and seedling dry weight, as well as accelerated aging, are used to evaluate seed vigor (Peske et al., 2012).

In many cases, the application of herbicides in agricultural crops may introduce these molecules in the food chain (Dong et al., 2016). This process takes place as a result of the accumulation of these compounds in structures used in food, e.g., grains and seeds (Springer et al., 2014). With the objective of ensuring food security, some countries have established maximum levels of residues of different herbicides that are allowed in these structures (Wang et al., 2008). This value varies between grains/seeds of different species (Dong et al., 2016).

The accumulation of residues in seeds or grains depends on the stage of application of the active ingredient, as well as on the time interval between application and harvest. Some herbicides are capable of translocating to plant parts with high metabolic activity, and such herbicides may translocate to seeds (Cessna et al., 2002). When the application is made at early stages and with high plant moisture content, a greater quantity of residue may be detected in seeds, in virtue of greater translocation, especially if the harvest is performed chronologically close to the application, thus preventing the metabolism of the herbicide (Cessna et al., 2002).

The hypothesis of this study is that the application of the herbicide 2.4-D in wheat negatively affects the physiological quality of the seeds, leading to accumulation of the residue of this molecule in these structures. Given the above, the aims of this study were to evaluate the physiological quality of wheat seeds and to determine the residue of 2.4-D in seeds of this species, depending on stage of application and herbicide rates.

MATERIAL AND METHODS

The region where the experiment was conducted has temperate climate, with well-distributed rainfall and hot summer; it is rated as type Cfa by the Köppen classification. The experiment was arranged in a randomized block design with four replications. The treatments were arranged in a factorial scheme: factor A consisted of three stages of application (flowering, soft dough and hard dough of the wheat crop) and factor B consisted of four rates of herbicide 2.4-D [0 (control), 504 g a.i. ha^{-1}, 1,008 g a.i. ha^{-1} and 2,015 g a.i. ha^{-1} of the commercial product (U-46 BR®, 806 g L^{-1})].

The soil of the area was classified as a sandy loam Red-Yellow Acrosol (Embrapa, 2006). The experimental units (plots) were composed of an area of 7.65 m² (5 m x 1.53 m). The wheat crop, cultivar Quartzo, was sown with a nine-row seeder/spreader, using 17-cm spacing between rows. The final population of wheat plants corresponded to 300 plants m^{-2}.

Fertilization for soil correction was performed through the interpretation of soil testing, using 260 kg ha^{-1} of fertilizer formulation 8-18-28 (N-P-K). For weed control, herbicide Hussar® (iodosulfuron-methyl) was applied at 14 days after emergence (DAE) at a rate of 100 g ha^{-1}.

During the phenological cycle of the crop, two applications were made of N in the form of urea, at 21 and 35 DAE; at each stage of application, 55 kg ha^{-1} was applied. Also, fungicide Fox® (trifloxystrobin + prothioconazole) was applied at a rate of 0.5 L ha^{-1} of the commercial product, at 48 and 74 DAE, adding oil Aureo® at a rate of 0.5 L ha^{-1}.

Stage of application was determined through visual observation of the phenological stage of the plants. It was also based on the determination of seed moisture content, carried out by randomly collecting 20 ears of the crop, within the area of the plot. After the seeds were threshed manually and milled, two subsamples of 5 g per replication of each treatment were placed in metal containers. After measuring the weight of the samples, they were taken to an oven at a temperature of 105 °C ± 3 °C for 24 hours. Then, moisture was calculated by using the difference in weight, based on seed moist weight. Thus, the moisture values of wheat seeds were approximately 55 and 39% when the herbicide was applied at the soft dough and hard dough stages, respectively (Table 1). By contrast, when application was performed at the stage of flowering, moisture content was not quantified because there were no seeds.

Herbicide application used a CO_2, pressurized backpack sprayer, calibrated with pressure of 30 psi, which provided a spray volume of 120 L ha^{-1}. The applications at the flowering, soft dough and hard dough stages corresponded to 86, 107 and 127 DAE, respectively.

Wheat was harvested in five central rows in each plot, with total usable area of 2.55 m². The samples were threshed in a plot thresher. As a result, moisture content was quantified for the produced seeds, using the methodology proposed by the Rules for Testing Seeds (Brasil, 2009).

Table 1 - Moisture of wheat seeds before (MBA) and after the accelerated aging test (MAP-AA), depending on the application of herbicide 2.4-D at different stages and rates/MAP (%)

Stage of application	Rate (g a.i. ha^{-1})	M_{AP} (%)[(1)]	M_{BA} (%)	M_{AP-AA} (%)
Flowering	0.0	-[(1)]	13.42	25.69
	504.0	-	11.63	24.97
	1008.0	-	11.94	23.72
	2015.0	-	13.22	24.64
Soft dough	0.0	54.96	13.15	25.88
	504.0	54.94	12.76	25.59
	1008.0	54.92	11.50	25.54
	2015.0	54.94	11.61	24.95
Hard dough	0.0	38.78	12.53	24.65
	504.0	38.79	13.02	24.06
	1008.0	38.82	11.88	25.33
	2015.0	38.53	11.86	25.62

[(1)] Seed moisture content at the application stage.

After the harvest, the seeds were taken to the laboratory for assessment of their physiological quality, through the following tests:

- Germination: conducted in eight replications of 50 seeds, with a total of 400 seeds per treatment. Two sheets of Germitest paper were used as a substrate. They were previously moistened with distilled water in a ratio of 2.5 times the weight of the paper, arranged in the form of rolls and kept in a germinator at a constant temperature of 20 °C. The evaluation was performed eight days after sowing, and results were expressed as percentage of normal seedlings.

- First germination count: carried out together with the germination test. The number of normal seedlings was counted on the fourth day after the test had been set up.

- Cold Test: conducted with four replications of 50 seeds for each treatment. After 50 seeds were placed on paper, Germitest paper rolls were prepared and placed in transparent polyethylene bags, sealed and kept in a refrigerator at a temperature of 10 °C for seven days. After this period, the rolls were transferred to a germinator and maintained under the same conditions of the germination test. The number of normal seedlings was evaluated at four days after sowing.

- Accelerated aging: conducted in plastic gerboxes (11 x 11 x 3.5 cm), on top of a galvanized wire mesh, which suspends the seeds and keeps them from contact with the water. Each box received 40 mL of distilled water, and the seeds were evenly distributed on the galvanized wire mesh, in order to form a single layer. Subsequently, the boxes containing the seeds were covered and placed in a BOD incubator at 41 °C, where they remained for 48 hours. After this period, the seeds were submitted to the germination test and evaluated at four days after sowing for number of normal seedlings.

- Total length of seedlings, shoot and root: the measures of length were taken with the aid of a millimeter rule at four days after sowing, and the results expressed in centimeters (cm).

- Total dry weight of seedling shoot and root: the seedlings from the analysis of length were separated into shoot and root, placed in paper bags and then died in a forced air circulation oven at a temperature of 60 °C to constant weight. After that period, the weight of the samples was measured in a precision scale, and the results were expressed in milligrams (mg).

- Seed moisture content: two samples of 4.5 ± 0.5 g each were taken from each treatment and subsequently placed in metal containers. The weight of the samples was measured with a precision scale; then the seeds were taken to an oven at 105 °C ± 3 °C. The samples were kept in the oven for 24 hours. After the drying period, the metal containers were kept in a desiccator to ambient temperature (25 °C); subsequently, seed dry weight was determined. Seed moisture was calculated by means of the equation $M (\%) = 100 (W - w)/(W - l)$, where M is moisture in percentage; W is the weight of the container covered with a lid and the moist seeds; w is the weight of the container with a lid and the dry seeds; and l, the weight of the container with a lid.

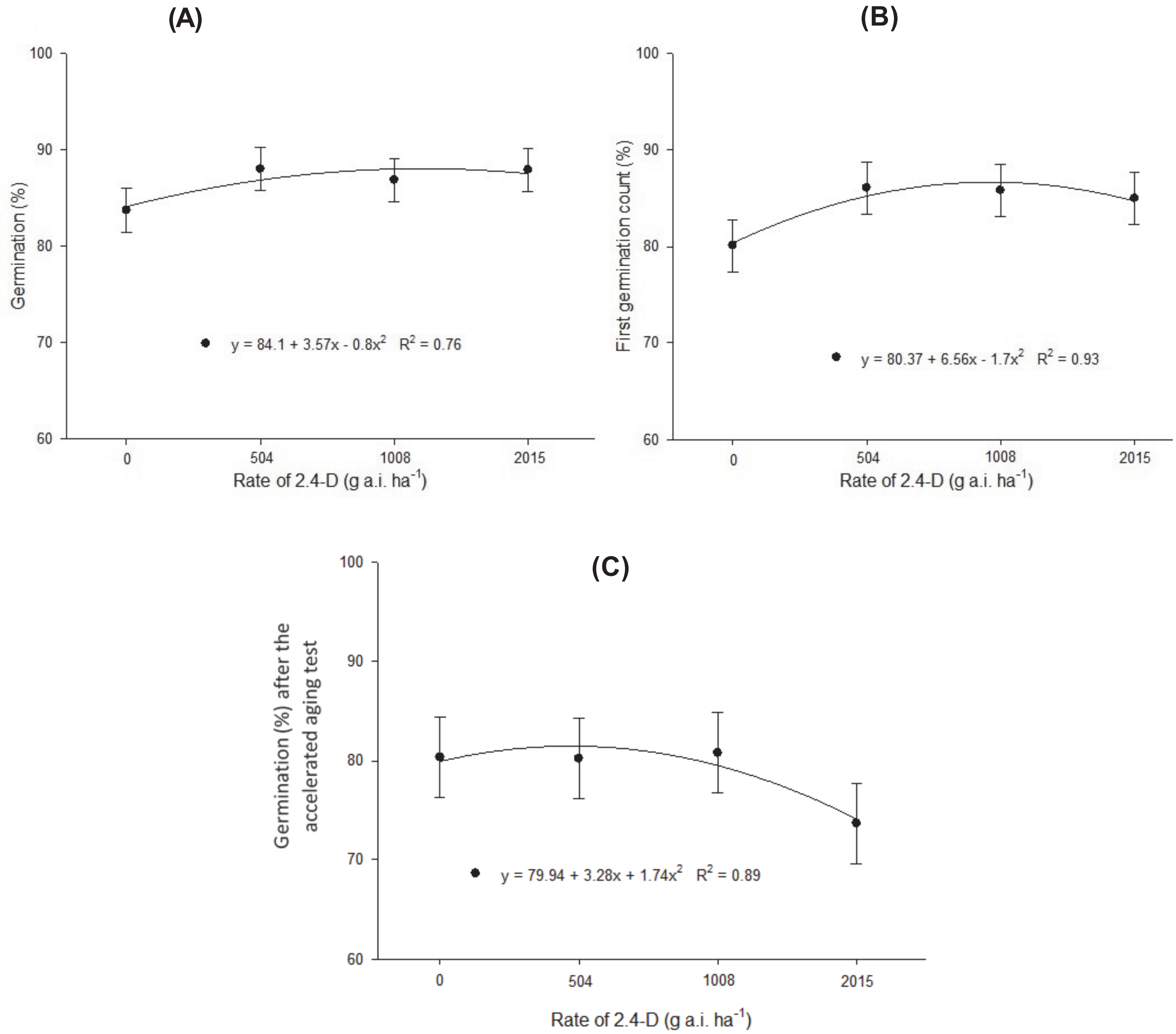
The residue of the active ingredient 2.4-D in wheat seeds was quantified by the company Bioensaios. For determination of residues, 20 mL of acetonitrile was added to 10 g of the processed and homogenized sample, and this sample was subsequently agitated in a vortex mixer. As a result, the pH value was adjusted to less than 5, and magnesium sulfate and sodium chloride were added. Later, the solution was agitated in a vortex mixer for one minute, and this aliquot was centrifuged at 3,000 rpm, for five minutes. As a result, an aliquot of the supernatant was collected. It was filtered out in a membrane filter (< 0.45 μm) and subsequently submitted to chromatographic analysis.

The data were submitted to analysis of variance by the F-test ($p < 0.05$); when significant, the factor stage of application was compared by Duncan's test ($p < 0.05$), while the factor rate was compared by polynomial regression analysis.

RESULTS AND DISCUSSION

There was no interaction between the factors for the evaluated variables. There was an effect of rate for the variables germination, first germination count and accelerated aging, while for total seedling dry weight, shoot length and total length, there was an effect of stage of application. As for the other variables, there was no effect of treatment (data not shown).

The germination test showed that the lower germination of the wheat seeds occurred in the seeds from plants that had not received herbicide 2.4-D (Figure 1A), but there were no differences for the other evaluated rates. The data on germination were adjusted to the quadratic model, and the point of maximum germination occurred at the rate of 1312.5 g a.i. ha^{-1}.



*The bars represent the confidence interval for the effect of rate.

Figure 1 **-** Germination (A), first germination count (B) and germination after the accelerated aging test (C) in wheat seeds from plants submitted to different rates of herbicide 2.4-D.

The standards for producing and marketing seeds of wheat (*Triticum aestivum* and *T. durum*), established by the Ministry of Agriculture, Livestock and Supply, determine that minimum germination must be 80% for seeds of these species to be marketed in the certified category (C1 and C2). In this way, it was found that the seeds produced, regardless of herbicide application rate, reached the minimum standards required for marketing, hence they can be included in the certified category.

By evaluating the effects of 2.4-D on the germination and respiration of seeds of barley and rice, it was found that seed germination for these two species was accelerated in the lowest tested concentrations of 2.4-D; when herbicide concentration exceeds a certain threshold, there was delay in seed germination for both species (Hsueh and Lou, 1947).

Synthetic auxins, such as 2.4-D, have herbicide activity when at appropriate concentrations, and when at low concentrations, they induce growth responses comparable to those of indoleacetic acid (IAA) (Kerbauy, 2008). Among the changes, growth regulators influence protein metabolism, and they may increase the rate of synthesis of enzymes involved in the process of seed germination (Mcdonald and Khan, 1983). In the process of germination, the auxins are involved in the permeability of the membranes and have a direct relationship with the growth of seedlings.

During the identification of physiological and biochemical alterations in seeds of different plant species that received the application of 2.4-D, it was found that the herbicide promotes germination when it is applied at low concentrations (0.01%) (Hsueh and Lou, 1947). However, the authors found that, when herbicide 2.4-D is applied at a concentration of 1%, it inhibits both aerobic respiration and seed germination.

When evaluating the effect of reduced rates of 2.4-D on the reproductive stage of soybean plants, it was found that it did not affect germination and initial growth of soybean seedlings (Neves et al., 1998). These authors found that the application of 2.4-D at the beginning of plant formation caused reduction by 8% in seed germination and 11% in hypocotyl length of soybean seedlings, compared with the application performed at the beginning of flowering.

In many cases, the occurrence of residue in seeds may cause negative effects on them, especially as regards germination-related processes. However, due to the fact that 2.4-D is a synthetic auxin, the occurrence of low levels in seeds may have possibly caused an increase in germination values, compared with the treatment that did not receive the application of this herbicide.

First germination count values were similar to those of germination values (Figure 1B). Thus, the smallest number of normal seedlings in the evaluation was found at rate zero of 2.4-D, i.e., when the herbicide was not applied. The values for first germination count also showed a quadratic tendency; the number of normal seedlings increased in the presence of the herbicide, and the largest number of normal seedlings was estimated by using a rate of 1562.5 g a.i. ha^{-1} (Figure 1B). It should be noted that, with the exception of the zero rate, there was no difference between the other rates, showing that increasing the rate does not compromise normal seedling development, as evaluated in the first germination count test for the seeds.

In the accelerated aging test, the highest percentage of normal seedlings was measured using the rate of 1.008 g a.i. ha^{-1} of herbicide 2.4-D. However, this rate did not differ from the lower rates (Figure 1C). However, the use of a rate of 2.015 g a.i. ha^{-1} caused a reduction in the number of normal seedlings by approximately 9% compared with lower rates.

The results found in the accelerated aging test can be checked by evaluating the moisture values of wheat seeds, measured before and after the seeds were exposed to aging (Table 1). For consistent results in the accelerated aging test, moisture of the seed samples should not have variation higher than 2.0% between the treatments to be evaluated (Marcos Filho, 2005). In this way, it was found that at all stages of application of herbicide 2.4-D, variation in seed moisture, before or after accelerated aging, was less than 2.0%.

When total dry weight, total shoot length and total length of wheat seedlings were evaluated, there was no difference between application at flowering and at the soft dough stage (Table 2). However, it was found that these three variables were reduced when the herbicide was applied at the hard dough stage. The application of 2.4-D in the hard dough stage reduced total dry weight, shoot length and total seedling length by 5, 6 and 4%, respectively, compared with the average of other application stages.

Table 2 - Total dry weight (DW_T), shoot length (L_S) and total length (L_T) of wheat seedlings, depending on the application of 2.4-D in wheat plants, at different stages

Stage of application	DW_T (mg)	L_S (cm)	L_T (cm)
Flowering	155 a[(1)]	10.35 ab	24.67 ab
Soft dough	154 ab	10.53 a	25.23 a
Hard dough	147 b	9.98 b	24.05 b

[(1)] Means followed by the same letter in the column do not differ by Duncan's test ($p < 0.05$).

The reduction of total seedling dry weight was due to the application of 2.4-D in plants; at the hard dough stage, it may be related to the amount of herbicide found in wheat seeds at the stage of application, in comparison with the other two application stages. Thus, the content of 2.4-D present in wheat seeds when the application was made at the hard dough stage possibly allowed the synthesis of ethylene, thus favoring the production of abscisic acid, which reduces the production of plant biomass by inhibiting growth through stomatal closure. As a result, carbon assimilation and, consequently, biomass production are limited (Kerbauy, 2008).

In plants, the main feature of auxin is induction of cell elongation (Kerbauy, 2008; Ohse et al., 2014). Thus, increased shoot length, found after application of 2.4-D at the soft dough stage, is a consequence of exposing the seeds to the appropriate hormone concentration. This allowed greater cell elongation in wheat seedlings at this stage of application.

Regardless of the rate tested, the seeds produced in the treatment where 2.4-D was applied at the soft dough stage had the largest accumulation of herbicide residue. This process was possibly due to increased metabolic activity of plants in this phase, as well as a consequence of the accumulation of reserve substances in seeds (Table 3).

The 2.4-D residue found when the herbicide was applied at the flowering stage was similar at all evaluated rates, and it was at the limit of quantification (Table 3). The occurrence of low levels of herbicide 2.4-D residue in seeds, by virtue of application at the flowering stage, was possibly due to the absence of these structures at the stage of application. It was also possibly due to the long time elapsed between herbicide application and residue determination.

Table 3 - Residue of the herbicide 2.4-D (R 2.4-D), as a function of different application stages of the wheat crop and rate of the herbicide

Stage of application	Rate (g i.a. ha^{-1})	R 2.4-D (mg kg^{-1})[(1)]
Flowering	0.0	0.0
	504.0	0.01
	1008.0	0.01
	2015.0	0.01
Soft dough	0.0	0.0
	504.0	0.03
	1008.0	0.03
	2015.0	0.14
Hard dough	0.0	0.0
	504.0	0.01
	1008.0	0.03
	2015.0	0.08

[(1)] Quantification limit of 0.01 mg kg^{-1}.

The wheat plants required a long period of time to metabolize the herbicide into secondary compounds. Moreover, 2.4-D may have been combined with sugars or amino acids, producing non-toxic molecules to the plants. In addition, the seeds had not yet been formed at this stage, hence the herbicide may have been translocated to other organs of the plant, or even exudated by the roots, as this is one of the mechanisms of selectivity used by auxinic herbicides.

The highest residual concentration of 2.4-D in seeds was found when the herbicide was applied at the soft dough stage (Table 3). Yet, at the same time, it was found that at the highest herbicide rate, compared with the lower ones, the residue increased by approximately 367% in the wheat seeds. During evaluation of the residual effect of 2.4-D in wheat and in the soil, it was found that the half-life of the herbicide is 1 day in wheat seedlings and 2.8 days in the soil (Liu et al., 2012).

When the herbicide was applied at the hard dough stage, the increased rate of 2.4-D resulted in a higher level of the residue in wheat seeds. The comparison between the lowest and the highest herbicide rates showed a 700% increase in the level of herbicide residue in the seeds. However, when the intermediate herbicide rate was compared with the highest rate, a 167% increase was found in the level of residue in wheat seeds.

In the literature, there are reports of studies where the application of 2.4-D in wheat caused the residue of the active ingredient to occur in seeds or grains of the crop (Cessna and Holm, 1994; Liu et al., 2012). In this sense, it was found that the application of 2.4-D in wheat, when the seeds were at the soft dough stage, resulted in a higher level of residue compared with applications made at the hard dough and ripening stages, in all plant structures evaluated (whole plant, seeds and straw) (Cessna and Holm, 1994). In addition, these authors found that increasing the herbicide rate caused an increase in the levels of residue in these structures.

When the beginning of the seed formation process is triggered, these structures become the preferential metabolic drain of plants until the end of the crop cycle, hence they require a high demand of assimilates. Thus, the largest level of residue of herbicide 2.4-D found at the soft dough stage, compared with the one at the hard dough stage, may possibly result from increased translocation of photoassimilates from the various plant structures to the seeds. Such increased translocation of assimilates may be related to quicker transfer of compounds in the initial phase of seed formation. In addition, this process is possibly associated with the longer period of time for compound translocation of the plants whose application occurred at the soft dough stage, compared with the application made at the hard dough stage, since the former (soft dough) was performed 20 days before the latter (hard dough).

Herbicides can be carried over to the seeds together with photoassimilates from other organs, resulting in the accumulation of substances in these structures. Sugars, amino acids and other solutes are transported from various plant organs to seeds via the phloem, through the osmotic gradient (Marcos Filho, 2005). Consequently, the highest level of 2.4-D residue was found when the herbicide was applied at the soft dough stage (Table 3), and it may be related to higher absorption and translocation of the herbicide, according to the largest photosynthetically active leaf area, compared with the hard dough stage. In addition, this process can also be due to the shorter time for metabolism of the herbicide when compared with the application at the flowering stage, as previously discussed.

In accordance with the standards of Brazil's Health Surveillance Agency (Anvisa), the Maximum Residue Limit (MRL) allowed for the active ingredient 2.4-D in wheat seeds and/or wheat grains is 0.2 mg kg^{-1}. It was found that, regardless of stage of application and rate of 2.4-D, the values of residue found in the seeds were below the MRL. Although the levels of residue found in seeds were below the maximum limit set by the health agency, regardless of the rate and stage of application being evaluated, the use of this practice leads to accumulation of herbicide residue in these structures.

The application of 2.4-D favors the physiological performance of wheat seedlings as regards germination and the first germination count; however, when application is performed at high rates and at the hard dough stage, it negatively affects total dry weight as well as shoot length and total length of seedlings. The application of 2.4-D results in accumulation of herbicide residue in seeds, especially when the treatment is performed at the soft dough stage. Moreover, increasing the herbicide rate increases the concentration of the residue. However, regardless of stage of application of 2.4-D and application rate in use, the values of residue found in the seeds are below the allowed maximum limit.

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