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Physiological and growth responses of lettuce plants submitted of 2,4-D simulated drift

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Abstract

Pesticide drift is due to the transport of the active ingredient to areas not targeted by spraying, causing negative effects on the sensitive crops' development. The aim of this study was to evaluate lettuce response to simulated 2,4-D drift. Experiments were conducted in the greenhouse, in a randomized complete block design with four replications. The treatments consisted of nine different doses of the 2,4-D herbicide, which are: 0; 1.56; 3.125; 6.20; 12.5; 25; 50; 75; and 100% from the recommended dose (670 g a.a. ha-1). The application occurred when the lettuce plants had four to six true leaves. The analyzed variables were visual injury (phytotoxicity), development parameter (phyllochron), growth (rosette diameter and dry mass of the aerial part), and photosynthetic parameters (gas exchange). The lettuce crop showed high sensitivity to the 2,4-D herbicide, with the lowest dose resulting in significant phytotoxic damage to plants, and from 12.5% of the recommended 2,4-D dose, visual injuries were greater than 90%. The plants´ development and growth were negatively affected by the increased drift doses, culminating in enhanced phyllochron and reduction in rosette diameter and dry mass of the aerial part. The gas exchange of the crop was affected from 6.20% of the recommended dose, causing a reduction in the CO_2 assimilation rate, stomatal conductance, transpiration rate and rubisco carboxylation efficiency.

Keywords: auxin mimics, horticulture, *Lactuca sativa L.*, photosynthetic physiology

Introduction

Lettuce (*Lactuca sativa* L.) is an herbaceous plant, belonging to the Asteraceae family and originally from the Mediterranean region. As it is the main leafy vegetable consumed in Brazil, lettuce holds about 40% of the total volume of commercialization of this group (Sala & Costa, 2012), playing a significant role in the national agriculture scenario. Most of the vegetables cultivated in the Brazilian territory is conducted in the conventional system, which means cultivation in the open fields. With the expansion of the agricultural area, farming vegetables and grains in nearby areas has become quite common, which can result in problems related to pesticide's drift, especially herbicides. In Brazil, there is a huge need to use chemical weed control due to the no-tillage system widely used in agricultural areas (Bottega et al., 2016). Among the most used herbicides, 2,4-D, stands out for its recognized efficiency and cost-effectiveness.

Herbicide drift through spraying can be defined as the movement of the spray droplets from the target area to areas where the application was not intended. Spray drift due to incorrect applications is the primary cause of herbicide drift. The secondary reason is related to soil losses and volatilization, which occurs up to days after application (Sharkey et al., 2021). Once herbicide drift reaches non-target plants, there may be a negative influence on the yield and morphology of sensitive crops. Research on the effect of 2,4-D in wheat has shown significant decrease in stomatal conductance values, photosynthetic rate, and transpiration when compared with plants that were not exposed to the herbicide (Agostinetto et al., 2016)

In general, 2,4-D is applied to control eudicotyledonous plants in pastures and weed desiccation in no-tillage systems, mainly to control glyphosate-resistant species (Craigmyle et al., 2013). Due to the mode of action of these herbicides, it is

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reported that drift situations have shown a high potential for damaging sensitive species, even in small quantities (Godinho Júnior et al., 2017). The matter about potential damage generated by the 2,4-D drift has been growing since the release of genotypes tolerant to this herbicide in some agricultural crops, such as soybeans and cotton, which increased its utilization (Mortensen et al., 2012). The objective of this study was to evaluate the effects of simulated drift of the herbicide 2,4-D on lettuce crop through the evaluation of the visual injury, development parameters, growth, and photosynthesis.

Materials and methods

Two experiments were conducted independently to obtain more accurate data regarding this study. The first experiment was conducted from June to July 2019, and the second experiment was conducted from August to September 2019, both in a greenhouse located in the Horticulture Sector of the Department of Crop Science at the Federal University of Santa Maria (UFSM). The city of Santa Maria is located in the Depressão Central physiographic region of Rio Grande do Sul state (latitude 29˚43'S, longitude 53˚43'W and 95m of elevation) from Brazil. The experimental design used was completely randomized, with four replications.

The lettuce seedlings used in the experiments were from cultivar 55 (Blue Seeds®), crisp lettuce group, obtained from 200-cell trays. When the seedlings had four true leaves, they were transplanted into four-liter pots, filled with sieved soil, classified as Sandy Loam Typic Paleudalf for the first trial. In the second trial, the containers were filled with a mix of 75% sieved soil and 25% commercial substrate (Mecplant®). Basic fertilization was performed on the transplant day, and two topdressings fertilizer at 14 and 21 days after the transplant, with doses based on soil analysis.

The treatment factor consisted of doses of the 2,4-D herbicide (U 46 D-Fluid 2,4-D®, Sumitomo Chemical, registered with the Ministry of Agriculture, Livestock and Supply –MAPA- of Brazil under number 4118103), as follows: 0; 1.56; 3.125; 6.20; 12.5; 25; 50; 75 and 100% of the minimum recommended dose for soybean pre-sowing sprayers, referring to 670 g a. i. ha^{-1}. The application was carried out seven days after transplanting the seedlings when the plants had four to six true leaves. The treatments were applied with a CO_2-pressurized backpack sprayer, at 200 kPa pressure, and equipped with a four nozzles bar, TeeJet DG Drift Guard Flat Spray Tip 110.015 type model, with medium drop size classification, spaced 0.5 m apart, calibrated to provide a 150 L ha^{-1} spray volume. The meteorological conditions at the spraying time were 12.8˚C, 77% relative humidity, and wind speed at 1.83 km h^{-1} at the first experiment. For the second one, the meteorological conditions were 16˚C, 81% relative humidity, and wind speed at 4.32 km.h^{-1}.

The analyzed variables were visual injury (phytotoxicity) (Frans, 1972) at 7, 14, 2,1 and 28 days after applications of treatments (DAT), phyllochron (development parameter), rosette diameter, and dry mass (growth parameters) at 28 DAT for both experiments. At the second experiment, photosynthetic parameters were evaluated at 24 and 72 hours after application of treatments (HAA).

Phytotoxicity was assessed visually using a percentage scale, which zero corresponds to an absence of injuries, and one hundred to death of plants. For the phytotoxicity variable, the data were adjusted to the sigmoidal logistic model, as follows:

$$Y= a/ [1+ (x/x_0)^b]$$

which: Y = phytotoxicity (%); x = dose of 2,4-D (%); a = difference between the maximum and minimum points of the curve; x_0 = dose of the herbicide that provided 50% of the response (ED50); b = slope of the curve.

From a simple linear regression (Y = ax + b) between the number of leaves emitted by the plants and accumulated thermal time (STa), the phyllochron was determined, as the inverse of coefficient *a* of the simple linear equation, that is:

$$\text{phyllochron} = 1/a$$

For thermal time calculation, the lettuce base temperature is equal to 10˚C (Brunini et al., 1976) and the maximum and minimum temperature values that occurred during the plant evaluation period were considered. Thus, the daily thermal time (STd, ˚C.day) was calculated according to McMaster & Wilhelm (1997):

$$STd=(Tmed-Tb).1day$$

where: Tmed = average daily air temperature (˚C); Tb = base temperature (˚C). The accumulated thermal time (STa, ˚C.dia) was obtained by adding the STd, that is:

$$STa=\Sigma STd$$

The number of leaves on the plants was counted weekly from the transplanting date of seedlings (7 days before the application of treatments) and throughout the experiment, at 0 (application's day of treatments), 7, 14, 21, and 28 DAT.

The rosette diameter was measured with a millimeter rule from the leaf's extremity to its opposite extremity, at 0, 7, 14, 2,1 and 28 DAT. The dry mass (DM) was determined at 28 DAT, when the plants were cut on the soil surface and placed in a dryer with air circulation until obtaining a constant mass, to finally weigh it. The results were converted to percentage of DM reduction related to the control (without herbicide application). The data were subjected to analysis of variance (ANOVA) and regression analysis when statistical significance occurred.

To assess the photosynthetic response of lettuce plants, evaluations were carried out 24 and 72 hours after application of treatments in the last fully expanded leaf, with an equipment Infra Red Gas Analyzer (IRGA), LI-COR brand, model LI-6400 XT. Evaluations were made between 9 and 11 am, to maintain homogeneous environmental conditions. Prior to physiological analysis, a light curve was obtained, which aims to define the compensation point and luminous saturation. The light saturation point was determined by varying the photosynthetic radiation (from 0 to 2000 μmol $m^{-2} s^{-1}$), which the plant reaches the maximum net CO_2 assimilation rate (A), in other words, from the saturation point, further increases in light intensity are not followed by an enhance in photosynthetic rate. Thus, the photosynthetic radiation of 1000 μmol $m^{-2} s^{-1}$ and the standard CO_2 concentration of 400 μmol mol^{-1} were used in the evaluations. The net assimilation rate of CO_2 (A) (μmol CO_2 $m^{-2} s^{-1}$), stomatal conductance (GS) (mol H_2O $m^{-2} s^{-1}$), transpiration rate (E) (mmol H_2O $m^{-2} s^{-1}$), and instant rubisco carboxylation efficiency (A/Ci) were evaluated. The data were submitted to regression analysis ($p<0.05$) with Sisvar statistical analysis system®.

Results and Discussion

The result of the analysis of variance showed statistical significance for all variables analyzed. The data adjusted satisfactorily to the models used. The results will be presented separately, as follows below.

Visual injury

The phytotoxicity results showed an enhance in the negative effects of 2,4-D on plants as the herbicide dose increased, regardless of the evaluation date for both trials (Figures 2 and 3). At 7 DAT (Figure 3A) it was observed that 100% of the 2,4-D dose caused 80% phytotoxicity and at the lowest doses, the phytotoxicity was around 10% (Figure 3A). From 14 DAT it can be identified that 25% of 2,4-D dose used was enough to promote 80% of phytotoxicity (**Figures 1**E and 3B), and from 21 DAT this level of injury showed 12.5% of the dose applied (Figure 3C). For the first trial, phytotoxicity was higher than observed on the second at initial evaluation dates, which differences can be due to season and growth conditions. However, similar results were observed at 28 DAT, with about 12.5% of the recommended dose, the phytotoxicity was greater than

Figure 1. Visual evaluation of 2,4-D drift in letuce at 14 DAT for the second trial: (A) Control x 1,56%; (B) Control x 3,125%; (C) Control x 6,20%; (D) Control x 12,5%; (E) Control x 25%; (F) Control x 50%; (G) Control x 75%; (H) Control x 100% from the recommended dose of 2,4-D. Santa Maria, RS, Brazil.

95% (Figures 2D and 3D). These results have shown that even at low doses, there was irreversible damage to the lettuce plants.

The evaluation of ED_{50} at 28 DAT has demonstrated the sensitivity of lettuce crops, which about 4% of the dose promotes 50% phytotoxicity to plants (Figure 2D and 3D) and may represent irreversible damage to the commercial product. These results are concern, since with the expansion of new crop technologies tolerant to auxin-mimicking herbicides and soybean fields (Mortensen et al., 2012), the 2,4-D application may be more frequent.

Development and growth parameters

The phyllochron variable (˚Cday.$leaf^{-1}$) showed that plants subjected to the highest doses of the herbicide 2,4-D needed a greater thermal time for new leaves emission, on average 79.14, 77.82 and 89.13 ˚Cday.$leaf^{-1}$, for treatments 50, 75 and 100% of the dose in the second trial (Figure 3E). Stands out that these values were

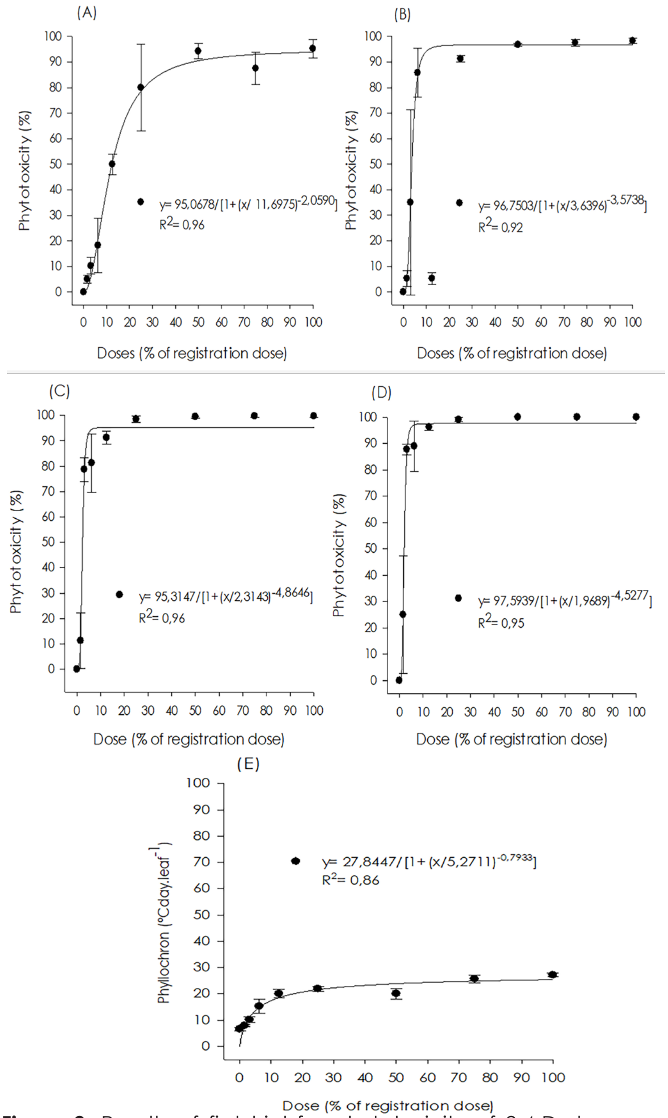

Figure 2. Results of first trial for phytotoxicity of 2,4-D doses on lettuce (Lactuca sativa L.) evaluated at 7 (A), 14 (B), 21 (C) and 28 (D) days after application and (E) Phyllochron of lettuce plants subjected to 2,4-D drift treatments. Santa Maria, RS, Brazil.

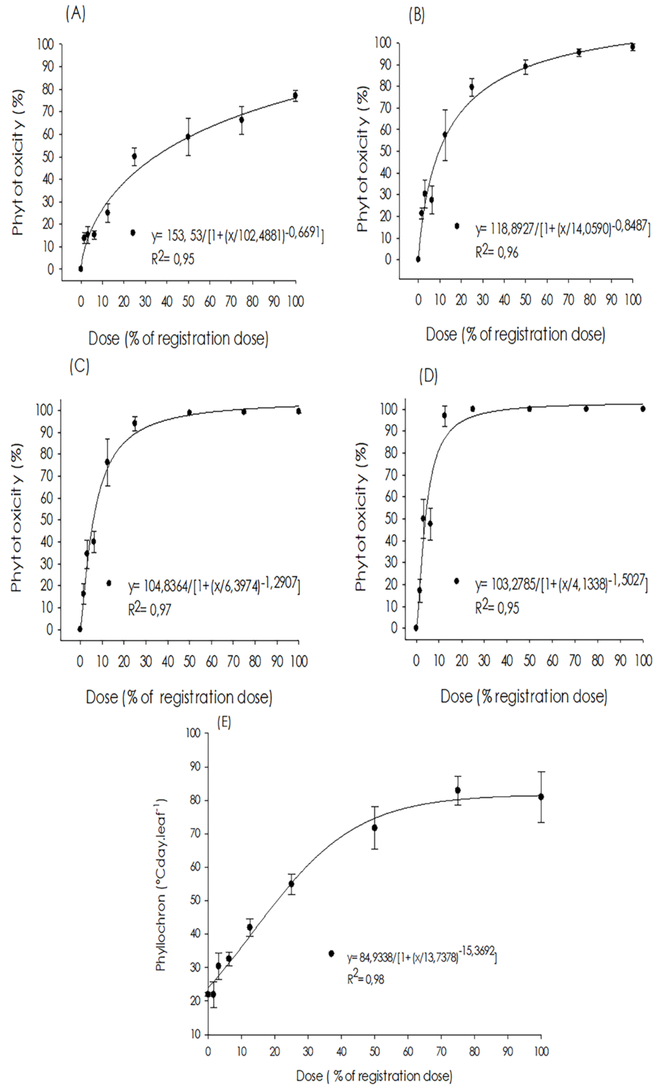

Figure 3. Results of second trial for phytotoxicity of 2,4-D doses on lettuce (Lactuca sativa L.) evaluated at 7 (A), 14 (B), 21 (C) and 28 (D) days after application and (E) Phyllochron of lettuce plants subjected to 2,4-D drift treatments. Santa Maria, RS, Brazil.

about four times higher than that observed in the control, which presented a phyllochron average, among the experimental units, equal to 21.94°C day.leaf^{-1}. Therefore, the higher 2,4-D dose on lettuce exposition, the slower will be its growth and development. In addition, the ED_{50} value indicated that about 14% of the 2,4-D dose increased the phyllochron by 50% (Figure 3E), specifically, the leaf emission rate becomes slow, suggesting that the herbicide drift effect delays the crop development. Similar results were found in Brazilian peppertree plants, where the simulated drift of 2,4-D and dicamba herbicides caused a high phytotoxicity even with low doses (Avila et al., 2022). For the first trial (Figure 2E), the thermal time needed for new leaves emission was even more significant, indicating that the plants stopped their development and consequently emission of new leaves due to the 2,4-D phytotoxicity effects.

Although the lettuce remained with active plant tissue at 7 and 14 DAT (Figure 3A and B, respectively), it is possible to notice the effect of the 2,4-D simulated drift by reducing rosette diameter from the first evaluation (7 DAT), when compared to the values obtained in the observation performed immediately before the treatment's application (0 DAT). At 14 DAT, the control treatment (0% of the recommended dose) obtained an average rosette size of 21.2 cm, while plants that received 100% of recommended dose obtained an average of 12.62 cm, illustrated in (**Figure 4**B) by the straightest slope, and figure 1 by the visual evaluation. The rosette diameter data for the first trial is not shown due to the reduced development and consequent death of plants at approximately 10 DAT. It is important to note that the experiments were conducted at different seasons, which may have influenced the difference in results.

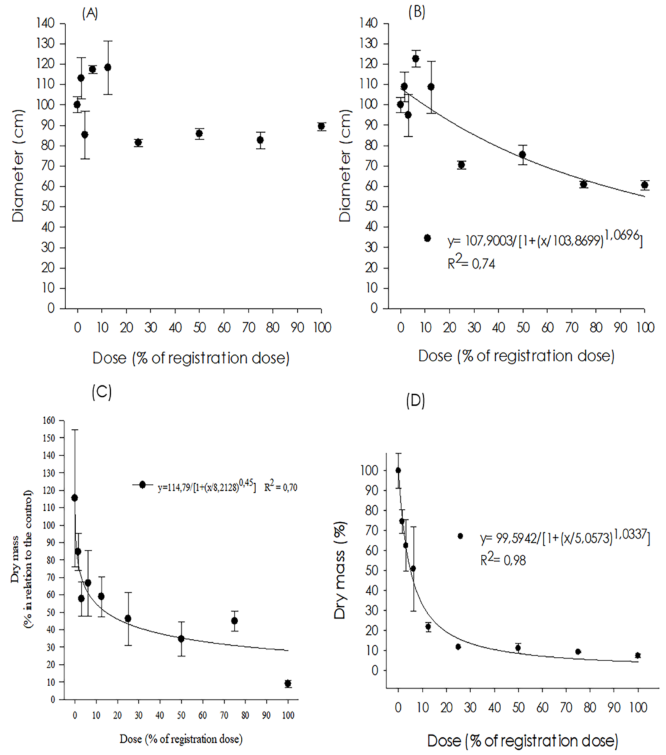

Figure 4. Results of second trial for (A) Rosette diameter on lettuce evaluated at 7 DAT; (B) Rosette diameter on lettuce evaluated at 14 DAT; Dry mass of lettuce plants (*Lactuca sativa* L.) after different application doses of 2,4-D for the first (C) and second trial (D), Santa Maria, Brazil.

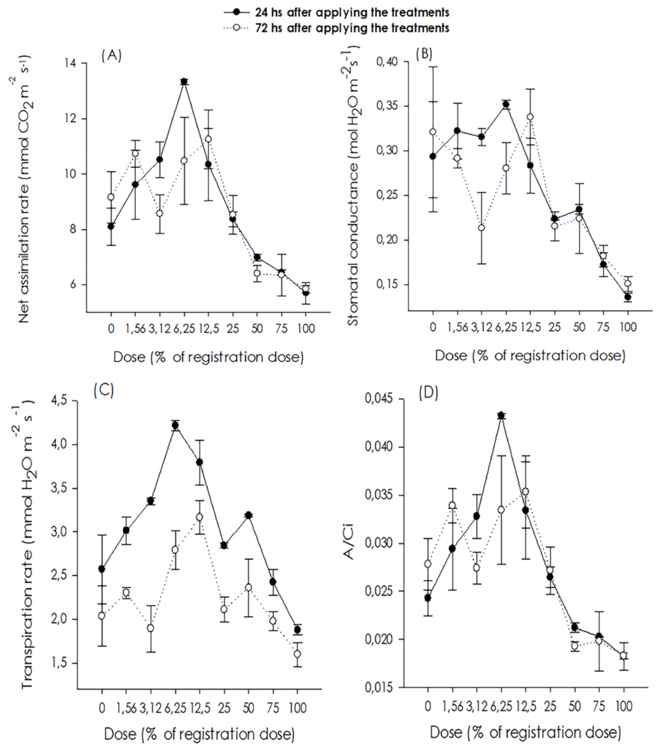

Figure 5. Results of second trial for CO2 net assimilation rate (A), stomatal conductance (B), transpiration rate (C) and instant efficiency of Rubisco carboxylation of lettuce lants (D) (Lactuca sativa L.), submitted to 2,4-D doses evaluated 24 and 72 hours after treatment's application.

For dry mass (DM) (Figures 4C and 4D), there was a reduction related to the control without 2,4-D application for all doses applied, showing the absence of overlap in the confidence interval even in the lowest doses. It is evident that from 1.56% (Figure 4C) and 12.5% (Figure 4D) of the recommended dose of 2,4-D a reduction of 80% or more in dry mass was reported in comparison to the control treatment. Thus, the 2,4-D herbicide drift can cause lettuce plant mortality and promote yield losses. The ED_{50} for the variable DM, was estimated at 5.05%, which represents the value which there is a 50% reduction in lettuce dry mass due to 2,4-D exposure (Figure 4D), like that observed for phytotoxicity at 28 DAT.

Photosynthetic parameters

After 24 HAA there was an increase in the rate of net CO_2 assimilation up to 6.25% of the 2,4-D recommended dose. Furthermore, there was a reduction in the variable, with 100% of the dose promoting a 35% reduction in the photosynthetic capacity of plants compared to zero doses (**Figure 5**A). At 72 HAA, the plants' behavior was similar to what occurred at 24 hours. For stomatal conductance in both evaluations, the increased recommended dose caused a Gs reduction, and at 100% of the recommended 2,4-D doses there was a reduction of 55 and 53% at 24 and 72 hours, respectively (Figure 5B). The reduction in stomatal conductance may have been caused by the 2,4-D action that stimulates both ethylene production and synthesis of abscisic acid in plants. Meanwhile, it accumulates initially in the leaves and then being translocated by the plant and acting on stomatal closure, which limits the net CO_2 assimilation rate and consequently reducing yield (Grossmann, 2010).

The transpiration rate decreased for both evaluations, from 12.5% of the recommended dose, and at 72 hours evaluation, the plants showed a more intense reduction. Hence, it can be directly related to A and Gs reductions because the stomata opening angle reduction difficult transpiration. Thus, auxinic herbicides can act on stomatal closure through less water absorption by the root system, reducing leaf turgor, leading the plant to reduce water losses with stomatal closure (Osipe et al., 20147). The instant efficiency of carboxylation in lettuce plants submitted to the 2,4-D application reported a similar behavior to what occurred for A, the A/Ci increased up to 6.25% and 12.5% of the recommended dose, at 24 and 72 hours, respectively. As the dose increased in the drift simulation, it caused a reduction in the variable (Figure 5C).

The 2,4-D herbicide has a high risk of drift and consequential damages to non-target crops, as can be seen in the present study with lettuce, where the phytotoxic effects of this herbicide can be considered severe, affecting photosynthesis, development, and growth of the plants. Therefore, its use should be avoided in areas close to susceptible crops in inadequate environmental conditions, which can increase the risk of drift. In this sense, measures can be adopted to reduce the herbicides drift, such as the selection of spray nozzles that provide larger drops, reduction of spray pressure, herbicide application in favorable environmental conditions with relative humidity above 55%, temperature below 30˚C, and wind speed below 10 km h^{-1}, adequate operating speed sprayer and. qualified applicator.

Conclusion

The simulated drift from 12.5% of the recommended dose of 2,4-D suggests phytotoxic damage above 90% after 28 DAT to lettuce plants. Lettuce development and growth are negatively influenced by the increase in 2,4-D doses, enhancing phyllochron, which consequently slows down plants' development. The crop gas exchange was affected from 6.20% of the recommended dose, causing a reduction in the net CO_2 assimilation rate, stomatal conductance, transpiration rate, and rubisco carboxylation efficiency.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.