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Articles

Simulated drift of 2,4-D and dicamba in pecan (*Carya illinoinensis* K. Koch) and olive trees (*Olea europaea* L.)

Deriva simulada de 2,4-D e dicamba em nogueira pecan (*Carya illinoinensis* K. Koch) e oliveira (*Olea europaea* L.)

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ABSTRACT

The cultivation of pecan (*Carya illinoinensis* K. Koch) and olive trees (*Olea europaea* L.) near pasture and grain cultivation areas in southern Brazil has caused herbicide drift problems. Therefore, the objective of this study was to evaluate the phytotoxic effects of herbicides 2,4-D and dicamba on the growth of pecan and olive tree seedlings. A total of 8 underdoses were used, as follows: 0, 1.56, 3.125, 6.25, 12.0, 25.0, 50.0, and 100% of the recommended doses of herbicides 2,4-D (670 g a.e ha^{-1}) and dicamba (720 g a.e ha^{-1}) for burndown. The applications were carried out in 80 cm-high seedlings with the aid of a CO_2 pressurized sprayer with a spray solution volume of 150 L. ha^{-1}. The analyzed variables were phytotoxicity at 7, 14, 21, 30 and 60 days after spray (DAA) and plant height and stem diameter at 30 and 60 DAA. The drift of hormonal herbicides caused damage to the growth of pecan and olive trees, decreasing their stem diameter and height, with the herbicide dicamba showing the greatest damage. In addition, doses above 12.5% of both herbicides resulted in phytotoxicity levels greater than 60%, and doses of 50 and 100% were responsible for leaf senescence.

Keywords: Underdoses; Herbicides; Phytotoxicity

RESUMO

O cultivo de nogueira-pecã (*Carya illinoinensis* K. Koch) e oliveira (*Olea europaea* L.) no Sul do Brasil próximo a áreas de pastagens e de cultivo de grãos tem registrado problemas de deriva de herbicidas. Diante disso, o objetivo do trabalho foi avaliar os efeitos fitotóxicos dos herbicidas 2,4-D e dicamba sob o crescimento de mudas de nogueira-pecã e oliveira. Para isso, utilizaram-se 8 subdoses, sendo elas 0; 1,56; 3,125; 6,25; 12,0; 25,0%; 50,0; e 100% das doses recomendadas para dessecação dos herbicidas 2,4-D (670 g e.a ha^{-1}) e dicamba (720 g i.a ha^{-1}). As aplicações foram realizadas em mudas com 80 cm de altura com auxílio de um pulverizador costal pressurizado a CO_2 com volume de calda de 150 L ha^{-1}. As variáveis analisadas foram a fitotoxicidade aos 7, 14, 21, 30 e 60 dias após a aplicação (DAA), estatura de plantas e diâmetro de caule aos 30 e 60 DAA. A deriva de herbicidas hormonais causou danos ao crescimento das plantas de nogueira e oliveira, diminuindo o diâmetro de caule e a estatura das mesmas, sendo o herbicida dicamba o responsável pelos maiores danos. Além disso, doses acima de 12,5% de ambos os herbicidas resultaram em fitotoxicidade superior a 60%, sendo as doses de 50 e 100% responsáveis pela senescência de folhas.

Palavras-chave: Subdoses; Herbicidas; Fitotoxicidade

1 INTRODUCTION

The state of Rio Grande do Sul (RS) in Brazil is known for presenting favorable conditions for the planting and development of fruit species that require the accumulation of cold hours for flowering and for good species yield, as is the case for pecan and olive trees. Belonging to the Jungladaceae family, the pecan nut tree (*Carya illinoinensis* K. Koch) is widely known to produce edible nuts and wood (Gatto; Haselein; Santini; Marchiori; Durlo; Calegari; Stangerlin, 2008). The United States and Mexico are the largest nut producers, accounting for 90% of the world's production, with an estimated production of 130,000 tons (INC, 2017). In Brazil, the state RS is the largest producer of nuts, with a total area of 2525 ha and a production of about 2.5 tons ha^{-1} (IBGE, 2019).

Introduced in the state of RS in mid-1948, the olive tree (*Olea europaea* L.) is known worldwide to produce olives that can be consumed *in natura*, and it is also used to manufacture olive oil (Cavalheiro; Rosso; Paulus; Cichoski; Wagner; Menezes; Barin, 2014). In the world, the largest producer is Spain, with 2,601,900 ha cultivated (FAOSTAT, 2019). In Brazil, 4015 ha are currently being grown, with an estimated production of 14,516 kg ha^{-1}.

Both species have been shown to be a good option for small properties since they can be intercropped with other crops and livestock (SEAPDR, 2017). Currently, they present profitable alternatives because there is a high economic value paid for their fruit. They have adapted well to the temperate climate, and the climatic conditions and favorable soils (EMBRAPA, 2018) make this species highly productive in this Brazilian state.

Although the cultivation conditions are conducive to the development of pecan and olive trees in southern Brazil, some characteristics have negatively impacted their yield, with the main one being the damage caused by herbicide drift. In many situations, the orchards of these species are located near pasture and grain production areas, where the herbicides used may be transported outside the spray target site.

Herbicide drift is characterized by the displacement of the sprayed product to nearby crops and occurs mainly due to the used drop size (generally less than 100 μm) (Godinho Júnior; Caixeta; Pereira; Ruas; Faria; Carvalho Filho, 2017), the action of the wind, temperature and relative humidity at the time of spray. The main reason for the drift of these compounds is an incorrect spraying method due to the use of an inappropriate spray tip, inappropriate height of the bar or inadequate application speed (Carlsen; Spliid; Syensmarck, 2006).

The use of auxin-mimicking herbicides is evident due to new technologies on the market (CTNBIO, 2017). The rapid evolution of resistant weeds has made it necessary to adopt new tools to control these plants. The use of new technologies will increase the use of auxiliary herbicides for the control of resistant weeds, thus increasing the risk of drift to adjacent areas cultivated with sensitive crops (Silva; Silva; Aguiar; Novello; Silva; Basso, 2018), such as fruit species in RS. The first year of use of the herbicide dicamba in tolerant cultivars in the United States promoted a series of reports of injuries to sensitive species outside the target spray area (Werle; Oliveira; Jhala; Proctor; Rees; Klein, 2018), suggesting the occurrence of drift.

The mode of action of auxin-mimicking herbicides involves a series of processes, with enzymatic activation and the expression of certain genes. At high doses, auxin-mimicking herbicides inhibit carbon assimilation and photochemical reactions (Grossmann, 2000), preventing electron transport to Photosystem II, decreasing carotene and chlorophyll concentrations, and increasing the xanthophyll/carotene ratio, causing plant senescence (Saygideger; Okkay, 2008). Understanding the impact of drift on pecan and olive trees is essential for defining the potential for damage to fruit trees under the growing conditions of southern Brazil. Based on the above, the objective of this research was to evaluate the effects of underdoses of 2,4-D and dicamba on the height, stem diameter and phytotoxicity in pecan and olive tree seedlings.

2 MATERIALS AND METHODS

The experiments were carried out in a greenhouse belonging to the Federal University of Santa Maria (UFSM) from July 2019 to February 2020, in a completely randomized design with four replications. Seedlings of pecan cv. Shawnee and olive cv. Koroneiki were acquired from nurseries registered with RENASEM and standardized at 50 cm in height. Pecan seedlings were kept in plastic containers with a capacity of 2 L and acclimatized in a greenhouse for 60 days until full sprouting and leaf expansion were observed. The olive seedlings were transplanted to pots with a capacity of 7 L containing duly sieved soil and fertilized with 100, 60 and 90 kg ha^{-1} P, K and N per plant, respectively, according to the fertilization recommendations of the species.

Irrigation was carried out daily, and the soil in the pots containing olive seedlings was maintained with a 75% water retention capacity. For this determination, the dry soil was weighed, saturated with water and a new weighing was carried out, defining the weight of each pot. The need for irrigation was determined with the aid of an analog scale, with each pot weighed daily and water added until the previously determined weight was reached. For pecan nut seedlings, light daily irrigation was carried out to maintain soil moisture. For both species, fertilization was supplemented monthly using urea at a dose of 100 kg N ha^{-1}.

The treatments were arranged in a 2 × 8 factorial scheme, where factor A was composed of the herbicides 2,4-D and dicamba and factor B was tested doses of the herbicides, which were 0, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100% of the recommended doses for burndown (670 and 720 g a.i. ha^{-1} for 2,4-D and dicamba, respectively). The treatments were applied on November 18, 2019, when the seedlings had fully expanded leaves, presenting an average height of 70 cm and an average stem diameter of 6.0 mm for pecan and 80 cm in height and 4 mm in diameter for olive trees. For this, a CO_2 pressurized sprayer was used, equipped with a 1.5-meter bar containing four tips of the XR Teejet 110.015 type at a pressure of 25 lbs in^{-2} and an spray rate of 150 L ha^{-1}. The climatic conditions at the time of application were 22.2°C, 67% and 2.5 m s^{-1} for temperature, relative humidity and wind speed, respectively. After spray, the seedlings were separated from each other, placed in an environment outside the greenhouse for 24 h, and returned to a controlled environment.

The analyzed variables were phytotoxicity assessed at 7, 14, 21, 30 and 60 days after spray (DAA) and plant height and stem diameter measured at 30 and 60 DAA, with the data corrected for percentage values of reduction in relation to the zero dose. Phytotoxicity assessments were performed visually using a percentage scale in which 0 indicates the absence of injury and 100 indicates the death of the plant (Frans; Talbert; Marx; Crowley, 1986). Plant height was measured using a millimeter ruler and counted from the soil surface to the node of the last fully expanded leaf of the plant. The diameter of the stem was measured with the aid of a digital caliper (Lorben stainless steel model 150 mm GT280).

The data were analyzed for normality and homoscedasticity using the Shapiro–Wilk and O'Neill–Matthews tests, respectively, showing that it was not necessary to transform the data. Subsequently, they were subjected to analysis of variance ($P \leq 0.05$) using the RStudio statistical software and the ExpDes package (Ferreira; Cavalcanti; Nogueira, 2011). When statistically significant differences were detected, regression analysis was performed using the quadratic polynomial model, as follows in Equation (1):

$$y = y0 + ax + bx2 \quad (1)$$

where: y = analyzed variable; x = dose of herbicide; y0 = maximum point of the curve; a = slope of the curve; b = minimum point of the curve. For this, the statistical program SigmaPlot® version 12.0 was used.

3 RESULTS AND DISCUSSIONS

3.1 Pecan tree

For the pecan seedlings used in the present study, 24 hours after herbicide spray, characteristic symptoms of auxin mimics were observed at the highest doses (100% of the recommended dose) and in young parts of the plants (Figures 1 and 2A, B). At 30 DAA, the main symptoms observed involved chlorosis and necrosis in the tissues of the youngest leaves (Figure 2C, D) for both herbicides, and they became senescent 60 DAA (Figure 2E, F). Consequently, the growth and development of the plants were negatively impacted.

For the phytotoxicity variable at 7 days, only the dose factor was significant, increasing the phytotoxic effect as the dose increased (Figure 1A). The interaction between the dose and herbicide factors was significant for phytotoxicity from the evaluation performed at 14 DAA (Figure 1B–E) and for stem diameter at 30 DAA (Figure 3C). For the plant height variables, regardless of the evaluation period and for the stem diameter at 60 DAA (Figure 3A, B, D), a simple effect of the herbicide dose factor was observed.

At 14 DAA, the spray of the herbicide 2,4-D promoted the greatest phytotoxicity in doses above 25%, the values of which were higher than the damage caused by dicamba (Figure 1B). After 21 DAA, there was an increase in phytotoxicity, reaching 90% at the highest doses applied (Figure 1C–E). For the lower doses (1.56, 3.125, 6.25, and 12.5%), the phytotoxicity values observed were close to 30% for both herbicides. For evaluations at 30 and 60 DAA, phytotoxicity increased with the increasing dose, in which doses higher than 25% of the recommended dose of both herbicides were close to 90% (Figure 1D, E). For the dose range between 1.56 and 12.5%, the percentages of phytotoxicity remained similar to those observed in previous evaluations, with values around 35%. Low doses of 2,4-D promoted morphological changes, such as decreasing leaf area and growth in plants from Pequi (Tavares; Pereira; Araújo; MARTINS; JAKELAITIS, 2017) and Uva 'Itália' (Oliveira Júnior; Constantin; Brandão Filho; Callegari; Pagliari; Cavalieri; Framesqui; Carreira; Roso, 2007), reinforcing what was found in this study. For 'Pokan' mandarin seedlings, foliar abscission caused by dicamba or 2,4-D at 720 and 2345 g a.e ha^{-1}, respectively, was around 20% at 7 DAA, and was 56% at 21 DAA for dicamba (Brochado; Mielke; Paula; Laube; Cruz; Gonzattoa; Mendes, 2022).

Figure 1 – Phytotoxicity (%) in *Carya illinoinensis* plants evaluated at 7 (A), 14 (B), 21 (C), 30 (D) and 60 (E) days after spray of the herbicides 2,4-D and dicamba

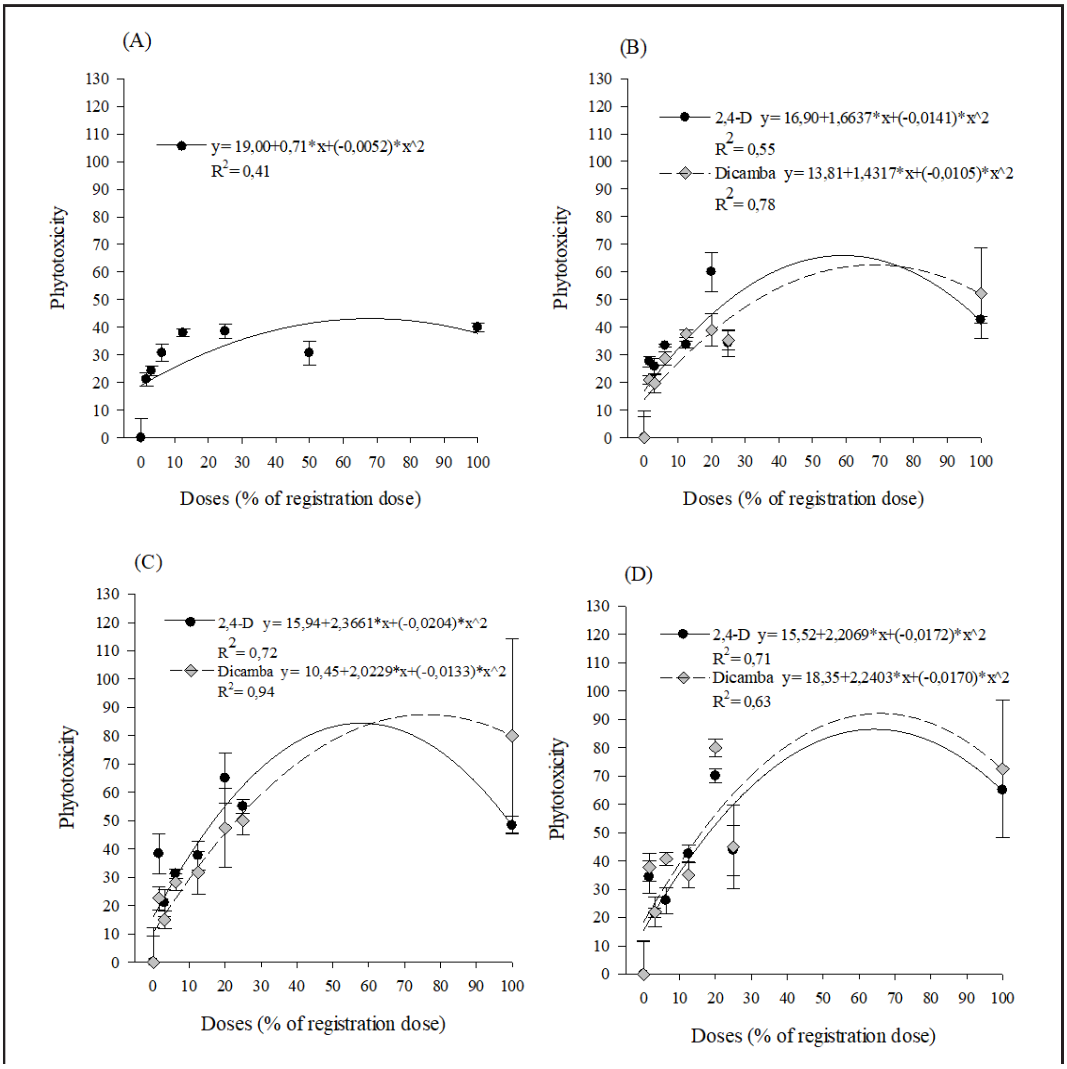

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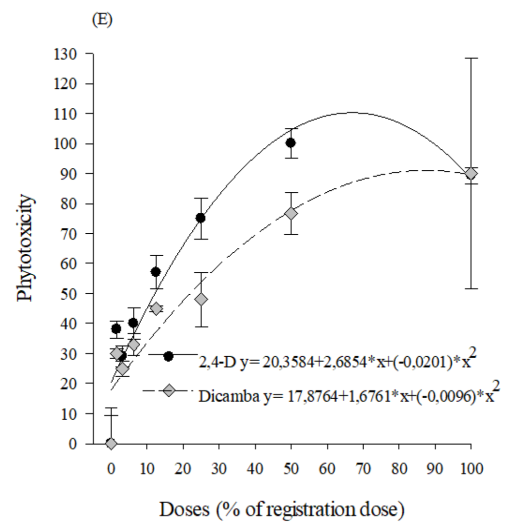
Figure 1 – Conclusion



Source: Authors (2021)

Even the smallest herbicide doses promoted injury to pecan nut trees up to 60 DAA (Figures 1E and 2E, F). As these herbicides are translocated by the xylem and phloem, even when applied in very low doses, they affect sensitive plants, causing damage to plant development and orchard yield. This may occur because the plants spend energy reserves to recover from the damage, not converting the photoassimilates to start sprouting and later fruit production, with sprouting being the most sensitive to the action of herbicides (Grossmann, 2010).

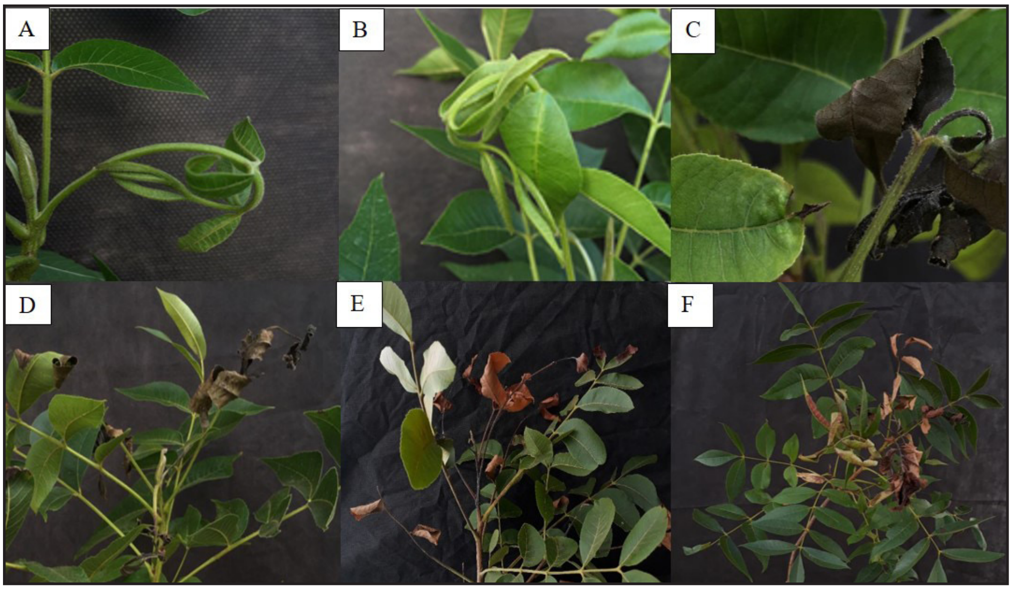
Thus, there was a reduction in plant height due to the increase in the herbicide dose at 30 DAA (Figure 3A), confirming the negative impact of these herbicides on crops. However, at 60 DAA, no adjustment of the model to the data was observed, although the absence of overlap in the confidence interval between the highest and lowest doses indicated a significant difference (Figure 3B).

Regarding the stem diameter, the values observed in the evaluation at 30 DAA demonstrated that the plants treated with doses higher than 25% of the herbicide dicamba showed the greatest reduction (Figure 3C). The values for this herbicide showed a 17% decrease in the plants treated with the highest dose compared to the control without spray. Glyphosate application in eucalyptus plants promotes a reduction in stem diameter; consequently, less wood is produced (Tuffi Santos;

Machado; Viana; Ferreira; Ferreira; Souza, 2007). As for herbicide 2,4-D, there was a small increase in the stem diameter of the plants that received up to 25% of the dose, and it can be inferred that there was no negative effect of the herbicide on the development of plant stems after its application.

In the evaluation carried out at 60 DAA, there was no interaction between the herbicide and dose factors; however, similar values were found between the applied doses (Figure 3D), showing that there was recovery of plant stem growth. The same was observed in eucalyptus plants when subjected to underdose glyphosate spraying, in which 35 DAA of the herbicide no longer showed growth damage (Pereira; Rodrigues; Campos; Melhorança Filho; Martins, 2011). The stem diameter evaluation infers the size and vigor of a tree when planted in a given location (Perdoná; Martins; Suguino; Soratto, 2012). Therefore, a smaller stem diameter makes it more sensitive since it has less resistance compared to others, is more susceptible to damage caused by wind and animals, and can be more easily broken. In addition, the decrease in the development of the plants can cause a delay in the implantation of the orchard and, consequently, a longer time needed to start nut production.

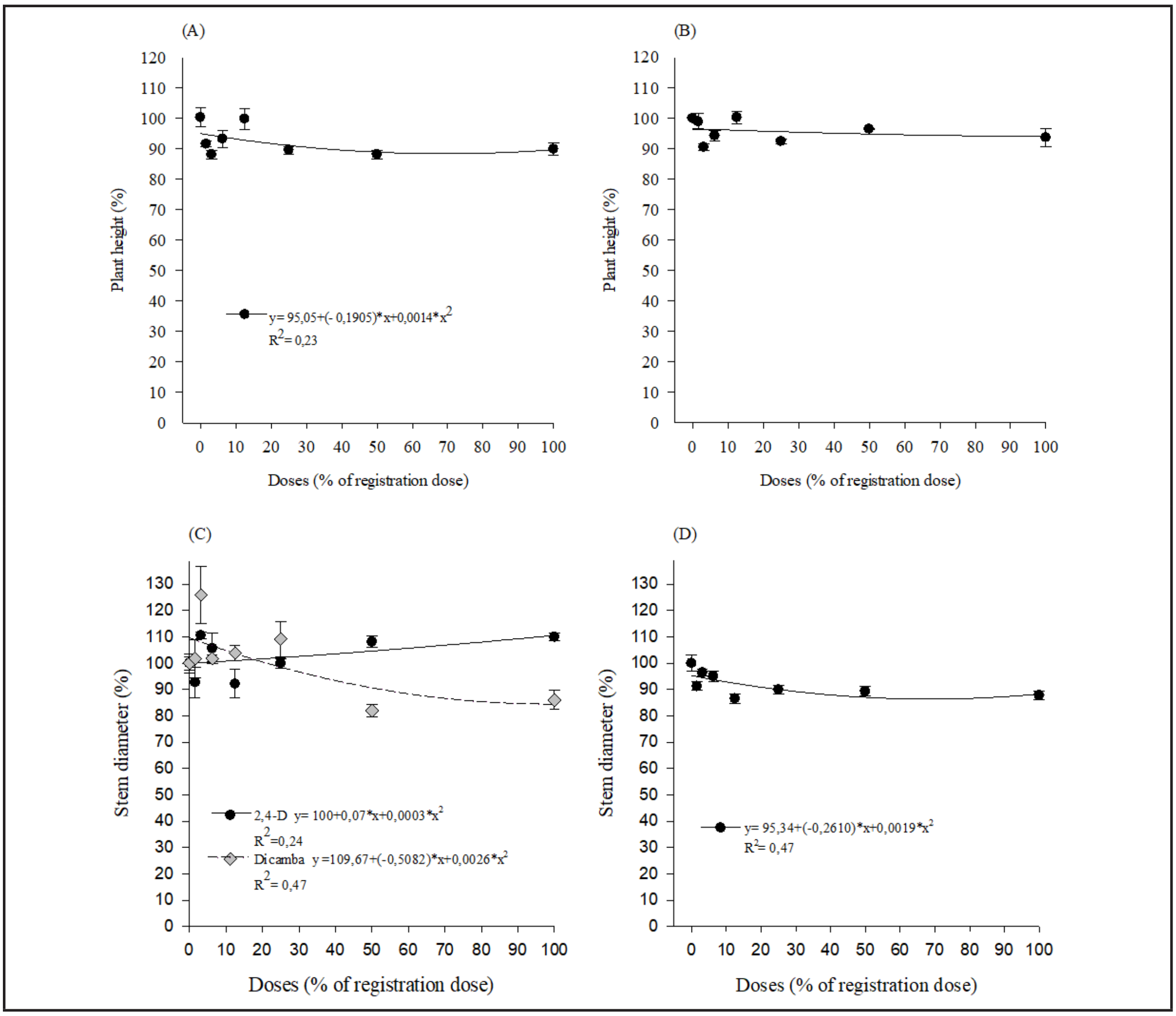
Figure 2 – Symptoms caused by the spray of the herbicides 2,4-D (A, C and E) and dicamba (B, D and F) 24 hours (A and B) and 30 (C and D) and 60 days (E and F) after the spray of 100% of the registered dose in *Carya illinoinensis*



Source: Authors (2021)

Based on the results obtained in this study, auxin-mimicking herbicides affect pecan trees, interrupting the natural regulatory processes of the plants through an uncontrolled response to auxin (Kelley; Riechers, 2007), consequently showing abnormal plant growth and affecting their production capacity. Therefore, it is possible to infer that the spray of these herbicides in crops near pecan tree orchards must be carried out following all precautions and guidelines since they may damage the development of these plants. Thus, complementary studies are necessary to verify if drift will cause damage to species' yield or if in more advanced stages of development, the damage will be as pronounced as it is in seedlings.

Figure 3 – Plant height and stem diameter (% in relation to the zero dose) of *Carya illinoinensis* measured at 30 (A and C) and 60 (B and D) days after the spray of the herbicides 2,4-D and dicamba



Source: Authors (2021)

3.2 Olive trees

There was an increase in phytotoxicity as the dose of both herbicides increased in all evaluations. At 7 and 14 DAA, doses higher than 25% were responsible for approximately 40% phytotoxicity. For the lowest doses (up to 12.5%), the values were around 25% at 7 DAA and 35% at 14 DAA (Figure 4A, B).

Figure 4 – Phytotoxicity (cm) in *Olea europapea* plants evaluated at 7 (A), 14 (B), 21 (C), 30 (D) and 60 (E) days after spray of herbicides 2,4-D and dicamba

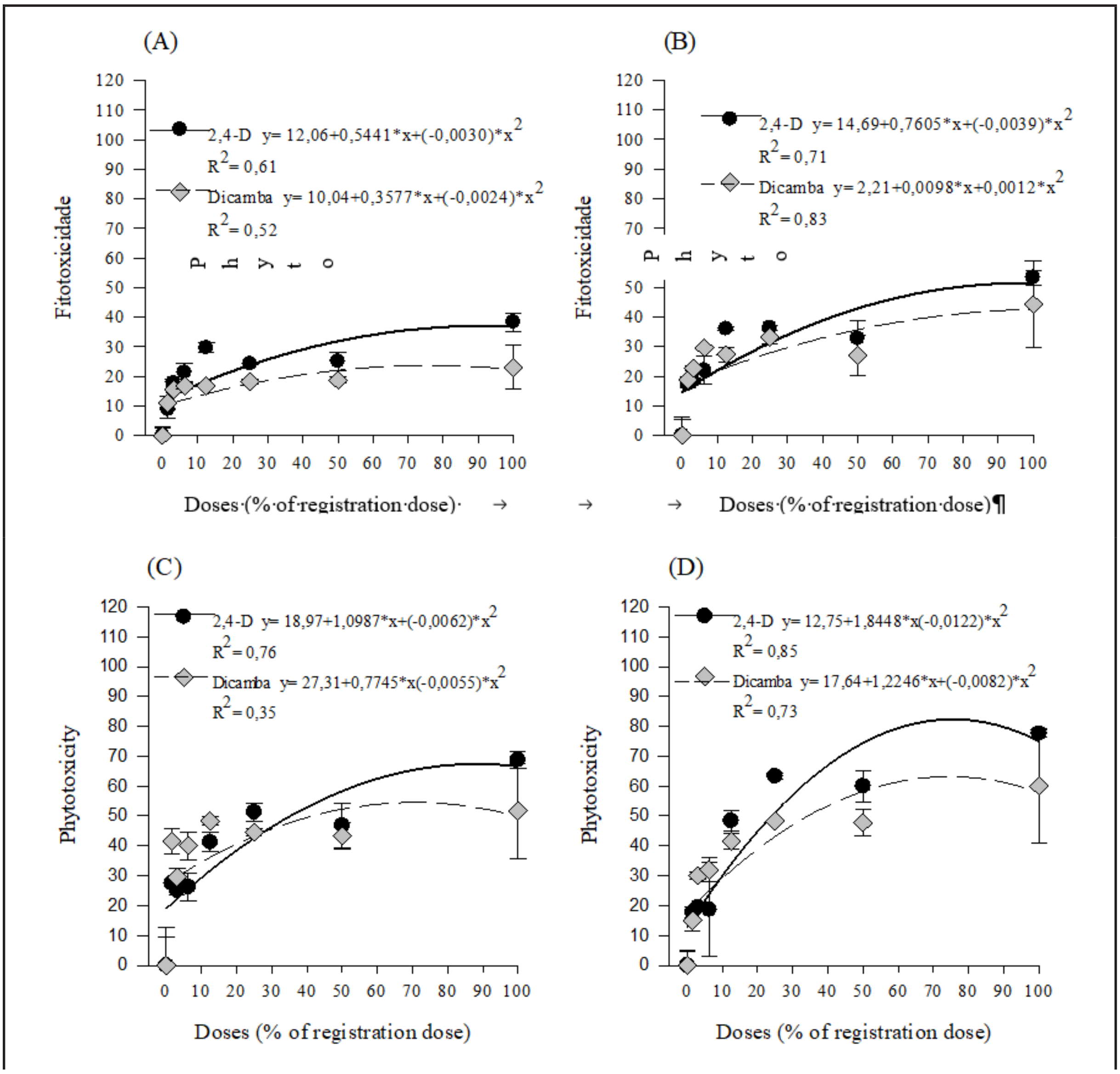


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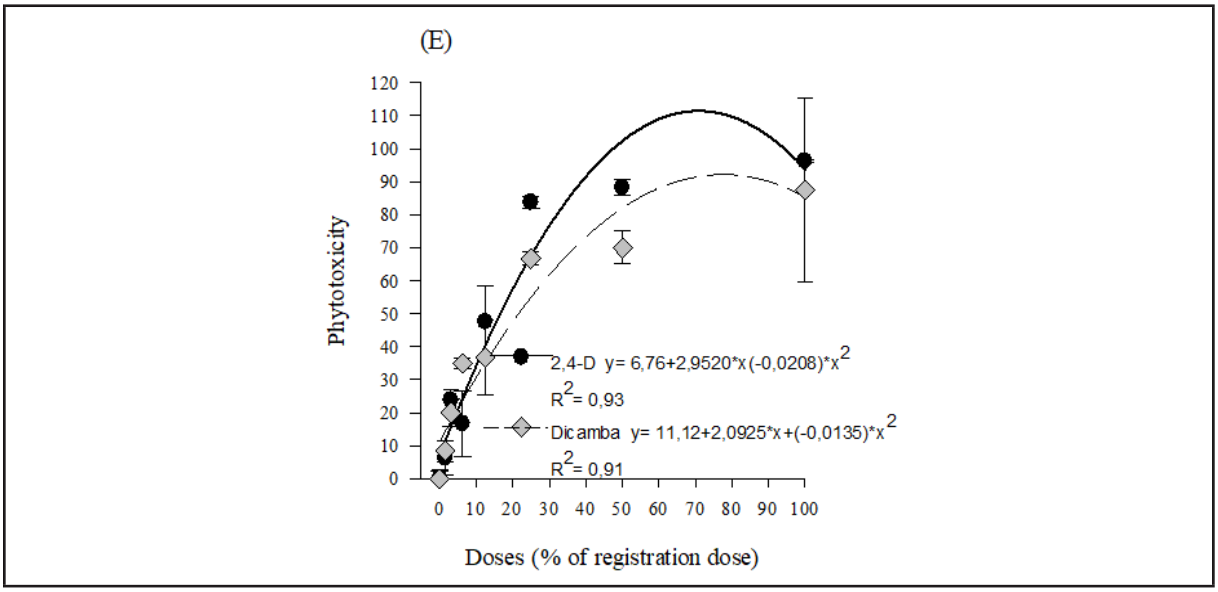
Figure 4 – Conclusion



Source: Authors (2021)

At 21 and 30 DAA, a dose of 100% obtained values close to 75% phytotoxicity (Figure 4C, D), showing that there was a significant increase in plant damage. This increase in phytotoxic effects on plants as the herbicide dose increases has also been observed in other plants, such as *Caryocar brasiliense* (pequi) (Tavares; Pereira; Araújo; Martins; Jakelaitis, 2017) and *Ceiba speciosa* (silk floss tree) (Monquero; Penha; Orzari; Hirata, 2011). In the evaluation performed at 60 DAA, 95% phytotoxicity was observed for the highest applied dose (100%) (Figures 4E and 5E, F).

Figure 5 – Symptoms present in *Olea europaea* plants 48 hours (A to D) and 60 days (E and F) after the spray of 100% of the registered dose of the herbicides 2,4-D (A, B and E) and dicamba (C, D and F)

To be continued ...

Figure 5 – Conclusion

Source: Authors (2021)

For doses of 25 and 50% evaluated at 60 DAA, the phytotoxicity values were close to 90% for herbicide 2,4-D and 70% for herbicide dicamba, showing that the greatest damage was caused by the herbicide 2,4-D and indicating the greater sensitivity of the species to this herbicide (Figure 4E). For the lowest doses (up to 12.5%), the values for both herbicides were close to 45%, indicating pronounced and persistent injury to olive plants due to the drift of these herbicides. These values showed that the plants did not recover from the damage caused by the herbicides and that the olive tree is an extremely sensitive plant to the mode of action of auxin-mimicking herbicides. In this sense, symptoms characteristic of auxin-mimicking herbicides were observed in the young parts of the plants 24 hours after spray with the highest doses (50 and 100%) in the present study (Figure 5A–D). At 60 DAA, the main symptoms observed involved chlorosis and necrosis in the tissues of the youngest leaves of both herbicides (Figure 5E, F).

For the plant height variable, a reduction in plant growth was observed as there was an increase in the applied dose. In the evaluation carried out at 30 DAA (Figure 6A), the zero dose showed values close to 95%, whereas doses above 12.5% presented values close to 85%, showing that the application of herbicides paralyzed plant growth. At 60 DAA (Figure 6B), this decreasing trend was maintained, resulting in values close to 100 cm for the smaller doses (up to 12.5%) and around 75% for the larger doses (25, 50 and 100% of herbicide doses). This reduction in plant height in relation to 30 DAA can be explained by the fact that the plants that received the highest doses underwent senescence, as shown in Figure 2. For both evaluations, herbicide 2,4-D was responsible for the lowest values obtained, showing that it can cause major problems in orchards.

The same pattern of reduction was observed for the stem diameter variable, in which, at 30 DAA (Figure 6C), the plants submitted to the zero dose obtained values of 100%, while the higher doses (50 and 100%) presented values around 95% for the herbicide 2,4-D. As for the herbicide dicamba, the plants treated with doses from 0 to 12.5% showed values of around 100%. Thus, it is evident that the lower doses of the herbicides did not cause damage to the development of the plants, and it is possible to observe a slight increase in values from the 25% dose, which can be explained by the fact that auxinic herbicides cause stem swelling (GROSSMANN, 2010).

Figure 6 - Plant height and stem diameter (% in relation to zero dose) of *Olea europaea* measured at 30 (A and C) and 60 (B and D) days after the spray of the herbicides 2,4-D and dicamba

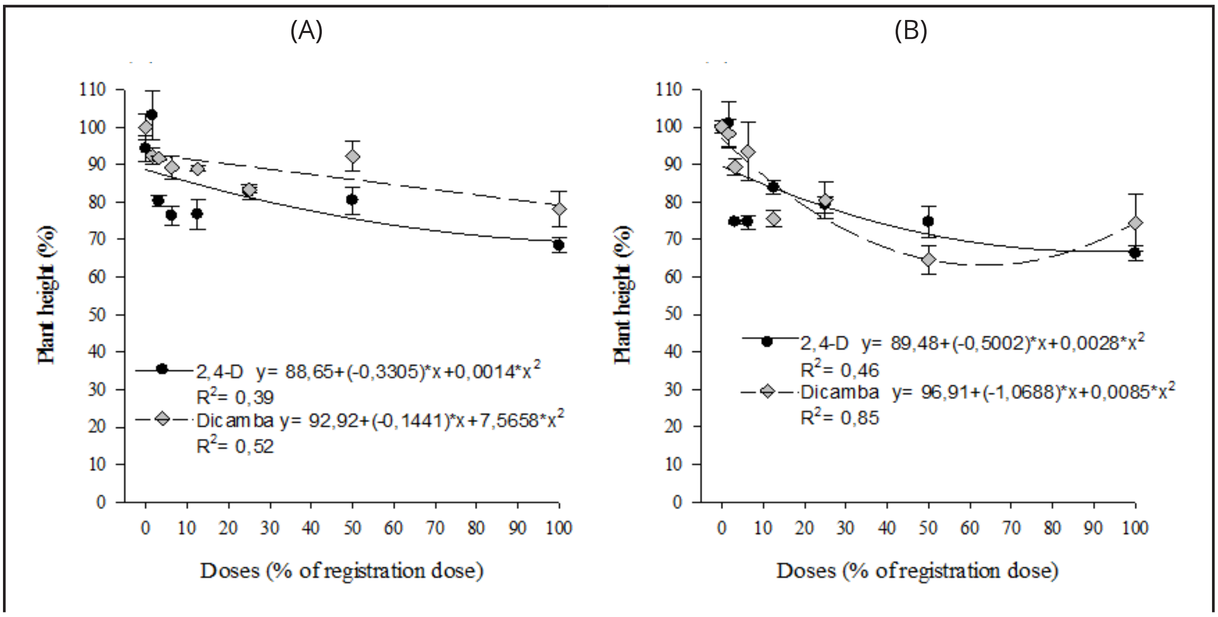


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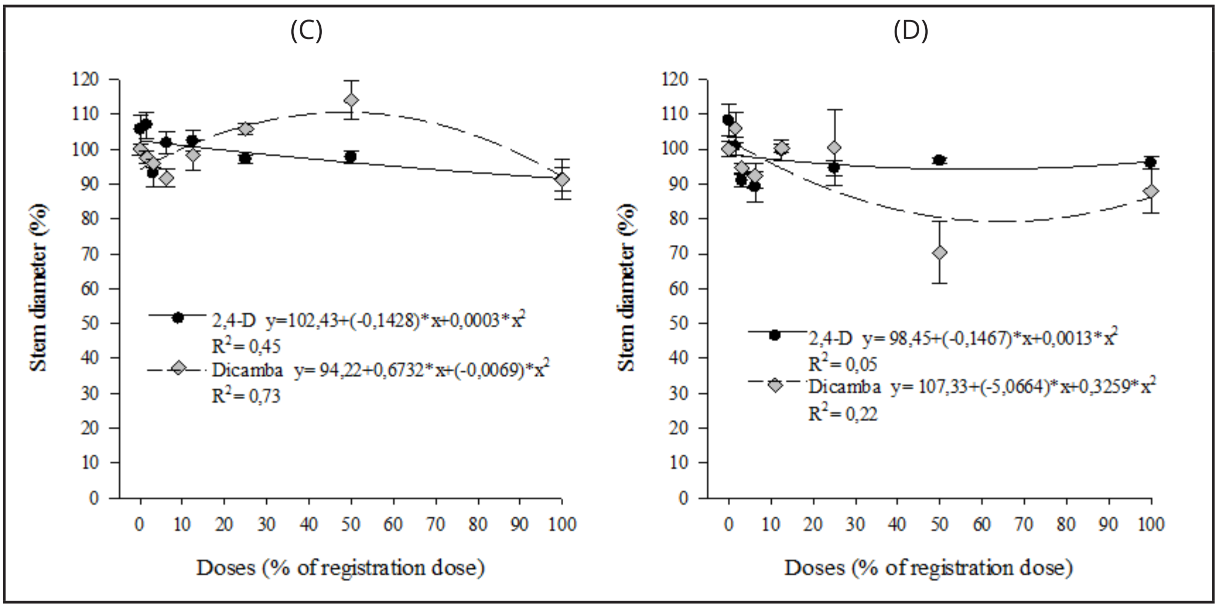
Figure 6 – Conclusion



Source: Authors (2021)

In the evaluation carried out at 60 DAA (Figure 6D), for the herbicide dicamba, which had previously had an increase in values, there was a decrease in the stem diameter of the plants, as there was an increase in the applied dose. At doses higher than 50%, the values obtained were around 90 mm. This is because, at 60 DAA, the plants were senescing, thus reducing the stem diameter. For herbicide 2,4-D, the values of all doses were very close to those of the zero dose, showing that the plants were already recovering from the damage caused by the herbicide.

Phytotoxicity was more pronounced in plants due to the fact that they were young plants and in higher doses of the products (Oliveira Júnior; Constantin, J.; Brandão Filho; Callegari; Pagliari; Cavalieri; Framesqui; Carreira; Roso, 2007; Ronchi; Silva; Terra; Miranda; Ferreira, 2005; Tavares; Pereira; Araújo; Martins; Jakelaitis, 2017), as shown in Figure 4E, F. In addition, even at low doses, the spray of the herbicides 2,4-D and dicamba caused damage to sensitive plants (Tavares; Pereira; Araújo; Martins; Jakelaitis, 2017), as is the case with olive trees, with the highest doses promoting a decrease in plant growth (Tavares; Pereira; Araújo; Martins; Jakelaitis, 2017). A similar finding was observed by Monquero, Penha, Orzari and Hirata (2011) after the spray of glyphosate, with a decrease in stem diameter and plant height.

4 CONCLUSIONS

The drift of auxin-mimicking herbicides causes damage to plant growth, showing high values of phytotoxicity to crops, even 60 DAA. Both pecan and olive tree species showed a reduced height and stem diameter at doses higher than 12.5% of the recommended dose for burndown with dicamba and 2,4-D in grain crops, indicating that plant growth was stopped for the species studied.

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