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Sensitivity of avocado seedlings to herbicides

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Abstract - An increase in crop productivity should be linked to the effective control of production costs. Thus, the chemical and selective control of weeds is an appropriate method because it saves labor and energy, requires little manpower, and allows control throughout the crop cycle. The present study aimed to evaluate the sensitivity of avocado seedlings to the phytotoxic effects of postemergence herbicide application. The experiment was carried out on avocado seeds of the cultivar Margarida in a greenhouse in the fruit sector of the Department of Agriculture, Federal University of Lavras. The experimental design was randomized block design, with four replicates and eight treatments, and each experimental plot consisted of three seedlings. The herbicides paraquat, oxyfluorfen, fomesafem, carfentrazone-ethyl, and glyphosate caused damage to avocado seedlings and should not be recommended based on the commercial dosage used. Paraquat caused the plants to die within 30 days after application. Fluazifop-p-butyl and imazethapyr proved to be the most promising for use in avocado orchards because they caused less damage to the crop.
Index terms: Chlorophylls. Management. *Persea americana* Mill. Phytotoxicity.

Sensibilidade de mudas de abacateiros a herbicidas

Resumo - O aumento na produtividade deve estar atrelado ao controle efetivo dos custos de produção. Assim, o controle químico e seletivo das plantas daninhas é um método adequado, pois proporciona economia de trabalho e energia, demanda pouca mão de obra e permite o controle durante todo o ciclo de cultivo. O presente trabalho teve como objetivo avaliar a sensibilidade da cultura do abacateiro aos efeitos fitotóxicos da aplicação de herbicidas em pós-emergência. O experimento foi instalado utilizando-se de sementes de abacate da cultivar Margarida e conduzido em casa de vegetação no Setor de Fruticultura do Departamento de Agricultura, da Universidade Federal de Lavras. O delineamento experimental utilizado foi de blocos ao acaso, com 4 repetições e 8 tratamentos e cada parcela experimental foi composta por três mudas. Os herbicidas paraquat, oxyfluorfen, fomesafem, carfentrazone-ethyl e glyphosate causaram danos às mudas de abacateiro e não devem ser recomendados com base na dosagem comercial utilizada. O paraquat causou a morte das plantas em 30 dias após a aplicação. Os tratamentos com Fluasifope-p- butílico e Imazetapir demonstraram ser os mais promissores para uso em área de implantação de pomares de abacateiros pois causaram menores danos à cultura.
Termos para indexação: Clorofilas; Manejo*; Persea americana* Mill.; Fitotoxidade.

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The Brazilian fruit market, an important sector of the national agribusiness, is considered complex and diverse. Avocado (*Persea americana* Mill.) is a high-value fruit in the global fruit market. Brazil is the sixth-largest producer, with 235,788 tons produced in 14,356 hectares, and this production is responsible for a revenue of approximately 318 million reais (FAO, 2018). According to the Institute of Agricultural Economics of the State of São Paulo (IEA), 97.9% of the national production was allocated to the domestic market, while 2.1% was exported to countries on different continents, demonstrating a strong internal demand and an increasing external demand for the fruit (BAPTISTELLA, 2019).

Perennial and semi perennial fruit plants require permanent crop management together with the management of the orchard environment. Consequently, their production cost is high when they seek to obtain good yields. Among the crop management practices, weed control, especially between planting rows, is important for the success of orchards (HAMMERMEISTER, 2016).

Adegas et al. (2017) state that among the various costs that go into avocado production, weed management deserves attention because if not properly controlled, weeds compete with the crop for water, light and nutrients and, may also be alternative hosts of pests and diseases, in addition to hindering crop management and harvesting operations. Chemical and selective weed control is the most appropriate method because it requires little manpower and allows more effective and, lasting control throughout the crop cycle, favoring higher productivity (SILVA; SILVA, 2007).

Selective herbicides are used as tools to eliminate weeds or at least delay their growth until the crop becomes dominant. Selective herbicides are those that control weeds without severely harming the crop of interest, and nonselective herbicides are those that affect both weeds and crops (OLIVEIRA Jr. et al., 2011). The indiscriminate use of herbicides can reduce productivity due to damage caused by phytotoxicity and increase the selection pressure of weed species resistant to the commonly used active ingredients. The lack of selective herbicides registered for avocado crops reveals the need for research to fill this gap.

Weed control in avocado nurseries and at the field-first years of the seedling is done manually, which requires a lot of labor and not always provides satisfactory results, or it´s done using herbicides that commonly cause phytotoxicity in a response to an application failure or drift problems. In the formation of avocado seedlings, conventional weed control methods are costly and time-consuming in a medium to large-sized nurseries and commercial orchards. Generally, in a year at least 2 or 3 weed control interventions are demanded. Currently, it is hard to think about weed management without herbicides. Thus, an ideal control would be one that economically eliminates the damage by weeds and does not affect avocado seedlings, which are very sensitive especially at this stage. In orchards that use chemical control is strongly recommended using herbicides without residual effect and soil activity; for this reason, glyphosate is practically the only one used in Brazil. It is common to use glufosinate-ammonium in other countries however, it should be noted that in Brazil this active ingredient is not registered to be used in orchards (SALOMÃO, SIQUEIRA, BORÉM, 2019).

At the present day, can be found two active ingredients registered by the Ministry of Agriculture, Livestock and Supply - MAPA, as a weed control tool in areas of avocado trees: glyphosate and diurom, both recommended in post-emergence application (MAPA, 2021).

In addition, the effect of herbicides in orchards is not always due to the local application, it can be derived from the pulverization drift of many neighboring cultures, which reinforces the importance of this work.

On this background, the present study aimed to evaluate the sensitivity of avocado seedlings to the phytotoxic effects of postemergence herbicide application.

The experiment was carried out in a greenhouse in the fruit Sector of the Department of Agriculture, Federal University of Lavras, Minas Gerais. The geographical coordinates of the area are a South latitude of 21° 14′ 06″, a West longitude of 45° 00′ 00″, and an average altitude of 910 m. The climate of the municipality of Lavras has two well-defined seasons, dry from April to September and rainy from October to March. Its climate is of the Cwb type under the Köeppen climate classification (REBOITA et al., 2015).

In this experiment, seeds of the avocado cultivar Margarida were used. They were collected from fresh fruits of donors-plants from the collection of the São Bento do Sapucaí Experimental Farm of the Council for Sustainable Rural Development of the state of São Paulo, located in the municipality of São Bento do Sapucaí. The seeds were selected according to (mean) size and phytosanitary conditions. Then, a third of the apical portion of each seed was cut, and it was planted in a black 5-liter polyethylene bag containing a mixture of three parts of sifted subsoil with a part of commercial substrate Tropstrato. The bags were kept on benches in a greenhouse with a 50% shading net for germination and the establishment of seedlings (PAIXÃO et al., 2016). The soil used as substrate was classified as a dystroferric red Latosol (EMBRAPA, 2018).

The experimental design was a randomized block design, with four blocks and eight treatments, where the control received distilled water, and each experimental plot consisted of three avocado seedlings at the field transplant stage—placed in black polyethylene pots for better organization— 8 months aged and ranging from 70 cm to 1 m in height. The treatments used in the experiment are listed in Table 1, including the name, applied dose, active ingredient, and formulation type of each evaluated product. During the testing, was not used fertilizers, biostimulants, fungicides, bactericides, acaricides, or another type of crop protection product. The treatments were applied in November 2018, on a day with no wind, relative humidity of 80%, and temperature around 27°C. Was used a manual backpack sprayer equipped with nozzles with a TT-11002 fan tip working at a height of 40 cm from the target with a spray rate of 1 $m \cdot s^{-1}$ and a spray volume of 200 $L \cdot ha^{-1}$. Two milliliters of INEX-A foliar adjuvant were added to each treatment. The herbicides were single-dose sprayed until complete wetting of the seedling's leaves surface.

Table 1. Description of herbicides (commercial name, active ingredient, formulation, and dose) applied to avocado seedlings (*Persea americana* Mill), Margarida cultivar.

Treatments (Commercial name)	Active ingredient (Common name)	Action mode	Group HRAC	Formulation	Dose (L cp/ha)
1. Control	-			-	-
2. Goal BR ®	Oxyfluorfen	Inhibition of protoporphyrinogen oxidase (PPO)	E	EC	2.0
3. Flex®	Fomesafen	Inhibition of protoporphyrinogen oxidase (PPO)	E	SL	0.7
4. Gramoxone 200®	Paraquat	Inhibition of photosynthesis in photosystem I	D	SL	1.0
5. Astral®	Glyphosate	Inhibition of the EPSPS enzyme	G	SL	2.0
6. Fusilade 250 EW®	Fluazifop-p- butyl	Inhibition of Acetyl CoA carboxylase (ACCase)	A	EW	0.7
7. Aurora 400 EC®	Carfentrazone- ethyl	Inhibition of protoporphyrinogen oxidase (PPO)	E	EC	0.05
8. Pivot 100 SL®	Imazethapyr	Inhibition of acetolactate synthase (ALS) or acid acetohydroxy synthase (AHAS)	B	SL	0.55

L cp/ha = liters of commercial product per hectare; EC = emulsifiable concentrate; SL = soluble concentrate; EW = oil-in-water emulsion.
Source: Author (2019).
HRAC (Comitê de Ação à Resistência de Herbicidas)

The visual evaluations of phytotoxicity to the crop were assigned scores on the EWRC scale (EWRC, 1964). The values range from 1 to 9, where 1 means no symptoms and 9 means plant death (Table 2). The evaluations were performed 1, 7, 15, and 30 days after the application of the herbicides (DAA).

Table 2. Evaluation scores and their corresponding descriptions of phytotoxicity.

EI	Description of phytotoxicity
1	No damage
2	Small changes (discoloration, deformation) visible in some plants
3	Small changes (chlorosis and curling) visible in several plants
4	Strong discoloration or reasonable deformation, without necrosis
5	Necrosis of some leaves, accompanied by deformation in leaves and shoots
6	Reduction in plant size, leaf curling and necrosis
7	More than 80% of the leaves destroyed
8	Extremely severe damage, leaving small green areas in the plants
9	Plant death

Source: EWRC (1964). EI: Evaluation index

The measurements of chlorophyll a and b were performed with a Clorofilog CFL 1030 FALKER portable chlorophyll meter in the middle and lower thirds of the plants, in fully expanded leaves (mature) and avoiding leaves with partial or total necrosis of the leaf blade.

Three evaluations were performed (7, 15, and 30 DAA), immediately after the phytotoxicity assessments.

Data normality was assessed using the Shapiro-Wilk test and the statistical analysis of the data was done using Sisvar software version 5.3 (FERREIRA, 2019). The means were compared among treatments by analysis of variance with the F test and then compared by the Tukey test at 5% probability.

After evaluating the sensitivity of avocado seedlings to the various products, there was a tendency toward greater sensitivity of the plants to two different mechanisms of action. Table 3 shows the mean scores of phytotoxicity caused to plants.

Table 3. Mean phytotoxicity scores of the treatments using the EWRC scale (1964), applied postemergence in avocados (*Persea americana* Mill.) seedlings of the Margarida cultivar obtained as the per-plot mean.

		Evaluations (DAA)		
Treatment	Dose (mL/L)	1	15	30
Control	-	1	1	1
Oxyfluorfen	10	1	6	7
Fomesafen	3.5	1	6	7
Paraquat	5	1	8	9
Glyphosate	10	1	7	8
Fluazifop-*p*-butyl	3.5	1	2	3
Carfentrazone-ethyl	0.17	1	6	6
Imazethapyr	2.75	1	2	3

DAA: Days after application.

It was expected that in 1 DAA, there would be no clear symptoms of phytotoxicity, especially for systemic herbicides that reach the sites of action through translocation. It is known that the translocation of herbicides can be influenced by their attributes, availability in absorption sites, the constitution of the plant surface, and environmental factors, so they usually do not show clear and apparent symptoms of plant-phytotoxicity at the first days after herbicide spray (MONQUERO, 2014). Therefore, contact herbicides, which do not translocate or have a minimal translocation can cause earlier severe symptoms of phytotoxicity (LINS et al., 2018).

At 7 DAA, the herbicide paraquat provided the loss of more than 80% of the leaves of the plants. There were no others that caused such severe damage in such a short time. Oxyfluorfen and carfentrazone-ethyl caused leaf curling and severe necrosis spots. The herbicides fomesafen and glyphosate, in some leaves, caused necrosis accompanied by small deformations, while imazethapyr and fluazifop-p-butyl triggered small changes with some chlorotic spots.

At 15 DAA, paraquat caused an increase in the damage index because of the emergence of extremely severe lesions, leaving little or no green area in the leaves. This product had the highest phytotoxic effect in this evaluation period, followed by glyphosate, which caused a considerable destructive effect on the plants, with more than 80% leaf loss. Plants that were sprayed with oxyfluorfen or carfentrazone-ethyl did not show damage evolution from the 7th to the 15th DAA. In avocados sprayed with fomesafen, at 15 DAA, phytotoxicity symptoms evolved, with curling and necrosis in the leaves. The plants that received fluazifop-p-butyl or imazethapyr also did not change from the 7th to the 15th DAA. These were the herbicides that provided the lowest damage scores to the plants.

At 30 DAA, the death of the plants that received paraquat was observed and this was the herbicide that caused the highest level of damage to avocados at the end of the evaluations. The plants under this treatment exhibited high sensitivity to the product in all evaluations starting at 7 DAA, evidencing the low or non-selectivity of this herbicide from the bipyridylium group. In general, herbicides from this group do not show selectivity. However, it is possible to use these herbicides selectively through postemergence-directed applications, in which the contact of the spray with the crop leaves is avoided (OLIVEIRA Jr. et al., 2011).

At 30 DAA, glyphosate, a herbicide of the glycine group, had the second-greatest toxic effect in this experiment because the plants under this treatment suffered extremely severe damage, leaving little or no green area. According to Oliveira Jr. et al. (2011) this herbicide is generally considered nonselective because of its broad spectrum, although it can currently be considered to selectively avoid genetically modified crops. The avocado seedlings under the oxyfluorfen and fomesafen treatments at 30 DAA showed destruction in more than 80% of their leaves. Both herbicides are inhibitors of the enzyme protoporphyrinogen oxidase and belong to the chemical group of diphenyl ethers, which may explain the similar behavior of plants exposed to these treatments. The plants treated with carfentrazone-ethyl did not change from the 7th DAA onward, showing some curling and necrotic spots in the leaves. The plants treated with fluazifop-p-butyl or imazethapyr showed the lowest visual phytotoxicity indices at 30 DAA. Only small spots of chlorosis were observed in the leaves. These results support the potential use of these two products in the crop row without causing severe damage to the seedlings in an establishing orchard.

Imazethapyr belongs to the class of ALS (acetolactate synthase) inhibitors, which can inhibit the synthesis of branched-chain amino acids (valine, leucine, and isoleucine), interrupting protein synthesis, which in turn interferes with DNA synthesis and cell growth (PETTER et al., 2011). The selectivity of this group of herbicides in some crops, such as soybean and wheat, is mainly based on the ability of plants to rapidly metabolize the herbicide into nontoxic forms (SWEESTER et al., 1982).

Fluazifop-p-butyl belongs to the group of acetyl-CoA carboxylase (ACCase) inhibitors, preventing lipid synthesis and interrupting the growth of treated plants (OHLROGGE; BROWSE, 1995). The selectivity of ACCase inhibitors for dicotyledonous species usually depends on the type of enzyme and its compartmentalization in the cell. The grass species have only cells with one form of ACCase shared by the cytoplasm and chloroplast stroma, while dicotyledonous species have a cytoplasmic enzyme equivalent to that of grasses, while the form present in chloroplasts is insensitive to these herbicides (SASAKI et al., 1995).

Paiva et al. (2015), in a study on the phytotoxicity of herbicides in passion fruit seedlings, evaluated 21 herbicides and observed the lowest phytotoxic effects for fluazifop-p-butyl and imazethapyr. Carvalho et al. (2014) also observed no phytotoxic effect of fluazifop-p-butyl when applied to coffee seedlings.

Chemical control is an important tool in the control of weeds in fruit trees, and it is common to apply the herbicides glyphosate, paraquat, and glufosinate-ammonium postemergence with spraying in the interrow of larger crops (banana, coconut, papaya). The application of these herbicides is considered efficient since they are broad-spectrum and nonselective products. What makes these herbicides suitable for use in fruit trees is the differential positioning: When sprayed among the rows, below the canopy of the orchards, the herbicides should not reach the cultivated plants, which makes their application selective (MOROTA et al., 2020).

Nascimento et al. (2016) evaluated the selectivity and efficacy of herbicides applied postemergence in the papaya crop and found that glufosinate-ammonium, paraquat, metribuzin, flumioxazin, ametryn, and the mixture fluazifop-p-butyl + fomesafen showed potential for use in papaya. The application of paraquat, ametryn, metribuzin, glyphosate and fluazifop-p-butyl + fomesafen requires protecting the stem of papaya.

The results of the visual sensitivity assessment are corroborated by those observed in the nondestructive analysis of photosynthetic pigments (Tables 4 and 5).

Chlorophyll evaluations were performed starting at 7 DAA, there were significant differences for the means of chlorophyll a and b, total chlorophyll and, the chlorophyll a/b ratios. The significant reductions in the mean chlorophyll levels followed the pattern of damage observed in the visual evaluations of phytotoxicity, making it evident that some of the herbicides used in this study are extremely harmful to the photosynthetic apparatus of avocados.

From 7th DAA to 30th DAA, plants that received paraquat, in general, had the minor average of chlorophyll a and b, except at 7 days when plants that received glyphosate had the chlorophyll b values statistically equal to those that received paraquat; at 15 days when the plants that received Fomesafen, Glyphosate, and Imazethapyr had the same average value of chlorophyll b as those that received paraquat; and at 30 days when plants sprayed with glyphosate had statistically the same value as those that received paraquat for chlorophyll a and b (Table 4). Regarding the total chlorophyll content and the chlorophyll a/chlorophyll b ratio, lower values were observed for avocado plants that received paraquat, except at 7 and 15 days, when there was no statistical difference between treatments for the ratio Cl*a/b*; and at

30 days when plants that received glyphosate had the same total chlorophyll mean as those that received paraquat and those sprayed with glyphosate and Fluazifop-p-butyl had the same Cla/b ratio value as those that received paraquat (Table 5).

Table 4. Mean chlorophyll *a* (Cl*a*) and chlorophyll *b* (Cl*b*) in leaves of the avocado (*Persea americana* Mill.) cultivar Margarida evaluated at 7, 15, and 30 days after herbicide application.

	7 DAA		15 DAA		30 DAA	
Treatments	Cl*a*	Cl*b*	Cl*a*	Cl*b*	Cl*a*	Cl*b*
Control	29.43 aA	15.60 aA	29.85 aA	14.10 aA	29.95 aA	13.90 aA
Oxyfluorfen	29.61 aA	16.70 aA	29.02 aA	13.12 aA	29.82 aA	15.60 aA
Fomesafen	30.16 aA	14.90 aA	29.15 aA	12.76 abA	27.66 aA	13.36 abA
Paraquat	12.65 bA	5.44 bA	13.16 bA	5.11 bA	0.00 cB	0.00 cB
Glyphosate	27.45 aA	12.47abA	28.23 aA	12.70 abA	5.40 cB	2.20 cB
Fluazifop-*p*- butyl	30.78 aA	14.64 aA	27.10 aA	14.60 aAB	26.50 aB	14.55 aB
Carfentrazone- ethyl	30.34 aA	13.22 aA	28.61 aA	13.50 aA	28.52 aB	13.30 aB
Imazethapyr	31.15 aA	15.10 aA	28.04 aA	11.43 abA	25.60 bA	12.34 bA

*Means followed by the same lowercase letters in the same column or uppercase letters in the same row do not differ statistically from each other at alfa=0.05 according to Tukey's test. The means are presented in Falker chlorophyll index (FCI) values. Source: Author (2019).

Table 5. Mean total chlorophyll (TC) and chlorophyll *a*/chlorophyll *b* ratio (Cl*a*/Cl*b*) in leaves of avocado (*Persea americana* Mill.) cultivar Margarida, evaluated at 7, 15, and 30 days after herbicide application.

	7 DAA		15 DAA		30 DAA	
Treatments	TC	Cl*a*/*b*	TC	Cl*a*/*b*	TC	Cl*a*/*b*
Control	45.02aA	1.90aA	43.94aA	2.19aA	43.24aA	2.20abA
Oxyfluorfen	46.31aA	1.88aA	42.14aA	2.27aA	39.90aA	2.26abA
Fomesafen	45.07aA	2.06aA	41.91aA	2.41aA	40.27aA	2.21abA
Paraquat	19.60bA	1.41aA	18.30bA	1.29aA	0.00cB	0.00cB
Glyphosate	41.17aA	2.03aA	38.43aA	2.14aA	7.62cB	0.60cB
Fluazifop-*p*-butyl	45.41aA	2.11aA	41.20aA	1.85aA	41.05aB	1.60bcB
Carfentrazone- ethyl	43.56aA	2.29aA	42.10aA	2.10aA	41.80aA	2.14abA
Imazethapyr	46.21aA	2.08aA	39.50aA	2.45aA	34.91abA	2.73aA

Means followed by the same lowercase letters in the same column or uppercase letters in the same row do not differ statistically from each other at $P<0.05$ according to Tukey's test. The means are presented in Falker chlorophyll index (FCI) values. Source: Author (2019).

All photosynthetic cells are composed of chlorophylls which are green pigments. Chlorophyll a (Cla) can be found in all organisms that uses oxygen to perform photosynthesis and it is crucial to carry out photochemistry (the first stage of the photosynthetic process), while the other pigments, known accessory pigments support the absorption of light and energy transference to the reaction centers, such as chlorophyll b (Clb) (STREIT et al., 2005).

According to Taiz; Zeiger (2013) at the photosynthesis first stage occurs the light energy conversion captured by the pigments of the antenna complex into chemical energy. This conversion is caused by the excited electrons transfer between pigments to the extant chlorophyll pair in the reaction center (P680) of photosystem II. The energized electron of chlorophyll proceeds through the electron transport chain, which will be conducted to photosystem I. This electron transport results in the chlorophylls of photosystem II being in an oxidized state, requiring the replacement of these electrons. Thus, could be certainly inferred that the reduction of chlorophyll levels negatively affects the potential plant photosynthesis consequently the production of photoassimilates.

These results demonstrate the greater toxic effect of this herbicide than the others. Given these results, further studies are recommended, including evaluating different doses of herbicides, to assess the possible phytotoxic effect associated with the use of inadequate doses.

The herbicides paraquat, glyphosate, oxyfluorfen, fomesafem, carfentrazone-ethyl caused damage to avocado seedlings and it should not be encouraged to use the commercial dosage recommended by the manufacturer's package insert, therefore they are not technically indicated for use in nurseries and newly formed orchards. The herbicides fluazifop-p-butyl and imazethapyr were the most promising for use in areas with newly established

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