

Research Article

Redroot pigweed interference with lettuce crop

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HIGHLIGHTS

- Density of 16 plants m⁻² of redroot pigweed, in the planting lines, most negatively affected lettuce growth
- Cultivar Lucy Brown showed greater tolerance to coexistence with redroot pigweed plants
- The cultivar Lídia was the most sensitive to the interference imposed by redroot pigweed plants

ABSTRACT

Background: Lettuce-production areas in Brazil are frequently infested by pigweed plants, which can interfere with production by competing for resources or releasing allelopathic compounds in the environment.

Objective: The present study evaluated the effect of redroot pigweed (*Amaranthus retroflexus*) density and distribution on lettuce crop (*Lactuca sativa* L.).

Methods: The cultivars Lídia, Verônica, Lucy Brown and Salad Bowl were used. After transplanting, these plants coexisted until harvest with four pigweed plants (equivalent to 16 plants m⁻²) located within or outside planting rows, or with one pigweed plant located at the center of the plot (4 plants m⁻²), as well as a control without weed. The experiment was conducted in an open and semi-controlled area, with treatments arranged in a 4x4 factorial scheme, in a completely randomized experimental design with three replicates. Evaluations included: the number of leaves, fresh matter (lettuce only), height, leaf area and dry matter of lettuce and redroot pigweed plants.

Results: The coexistence with redroot pigweed caused yield losses of up to 45% for cultivar Lídia; 41% for Salad Bowl; 33% for Verônica; and 28% for Lucy Brown.

Conclusions: The density of 16 plants m⁻² of redroot pigweed was the one that most negatively affected the growth of the cultivars, in which the positioning in the planting lines resulted in greater interference to the crop growth. Among the cultivars tested, Lucy Brown showed greater tolerance to coexistence with redroot pigweed plants, while the cultivar Lídia was the most sensitive to the imposed interference.

1 INTRODUCTION

Lettuce (*Lactuca sativa* L.) stands out in Brazil for its production and consumption. In 2012, its production reached more than 1.6 million tons, moving around R\$ 8 billion in retail alone. It became then the most

consumed leafy vegetable in the country (ABCSEM, 2013). São Paulo State stands out in this context. Of a total of more than 90,000 ha⁻¹ of lettuce area planted in 2018, this state accounted for about 11,800 ha⁻¹, with average yield of 19.7 kg ha⁻¹ (ABCSEM, 2013; BRASIL, 2013; CEAGESP, 2019; IEA, 2019).

In 2017, total market sales of this vegetable were approximately 49,400 tons in São Paulo State. The lettuce cultivars and types most traded in CEAGESP were 'Americana' (47%), 'Crespa' (38.5%), 'Mimosa' (5.9%) and 'Lisa' (4.28%) (CEAGESP, 2019).

The presence of weeds in lettuce-production areas interferes with production as they compete with cultivated plants for water, space, light, and nutrients. Weeds may also release allelopathic compounds in the environment (Blanco, 1983; Pitelli, 1984). In addition, weed communities can act as hosts for pests and pathogens, interfering with harvesting (Blanco, 1983).

Several weeds interfere with lettuce growth, such as: smooth pigweed (*Amaranthus hybridus*), jamaican crabgrass (*Digitaria horizontalis*), nutgrass (*Cyperus rotundus*), milkweed (*Euphorbia heterophylla*), and benghal dayflower (*Commelina benghalensis*) (Santos et al., 2003; Giacotti et al., 2010; Nascimento et al., 2013). Santos et al. (2003) emphasized that plants of the genus *Amaranthus* interfere with early lettuce growth due to light interception by their taller canopy, which makes it an important genus in lettuce cultivation areas. These weeds can decrease the quality of lettuce plants by reducing the head firmness, leaf size (Roberts et al., 1977) as well as nitrates and carotenoids content (Giannopolitis et al., 1989), leading to yield losses (Giacotti et al., 2010; Otero and Wright, 2013; Parry and Shrestha, 2018).

In this sense, given the economic importance of this crop, studies that aim to identify cultivars that are more tolerant to the competition imposed by relevant weeds in commercial areas have great relevance. These studies can help to direct the most suitable cultivars to areas with a predominance of infestations, thereby reducing yield losses.

The level of weed interference with the crop depends on several factors, which may be linked to the crop itself (such as cultivar used and planting spacing), the weed community (such as infestation density, distribution in the area, and species), the environment (edaphoclimatic conditions of the region), and the period of coexistence between weeds and cultivated plants (crop development stage and duration of competition) (Bleasdale, 1960; Pitelli, 1985).

Regarding weed-related factors, it is noteworthy that pigweed (*Amaranthus* sp.) is the main weed infesting lettuce-production areas (Kissmann and Groth, 1999), being an important species for

interference studies in lettuce crop (Shreffler et al., 1996; Santos et al., 1997, 1998, 2004a, b).

Thus, this study evaluated the effect of pigweed (*Amaranthus retroflexus*) density and distribution on lettuce crop (*Lactuca sativa* L.).

2 MATERIALS AND METHODS

An experiment was conducted in an open area without water restriction, in Jaboticabal city, São Paulo State (altitude of 590 m and geographical coordinates of 21°15'7" S and 48°19'20" W). The average air temperature during the experimental period was 23.1 °C (maximum of 29.9 °C and minimum of 17.5 °C), with an average relative humidity of 75.7%, and monthly insolation of 248 hours.

We used 75-L cement pots (0.5 x 0.5 x 0.3 m) previously filled with Dark Red Latosol with the following chemical characteristics: pH (CaCl₂) 6.0; 42 g dm⁻³ O.M.; 184 mg dm⁻³ P_(resin); V = 84%; 2.8, 79, 30, 22, 111.8, and 133.8 mmol_e dm⁻³ of K⁺, Ca²⁺, Mg²⁺, H⁺Al³⁺, SB, and T, respectively.

In order to evaluate the effect of weed competition on lettuce crop, we used pigweed (*Amaranthus retroflexus*). For that, we collected seeds from infested areas in Jaboticabal city, São Paulo State. Then, we sowed the seeds in a 128-cell styrofoam tray containing substrate (Plantmax[®] HF), and transplanted them into the cement pots at 18 days after sowing. Plant density and planting arrangement varied according to the experimental treatment.

Lettuce (*Lactuca sativa*) cultivars were as follows: Verônica, Lídia, Lucy Brown, and Salad Bowl. We obtained lettuce seedlings from vegetable markets in Jaboticabal-SP. These seedlings had, on average, four leaves at the time of transplanting (23 days after sowing). Spacing was 0.3 x 0.3 m, allowing four lettuce seedlings of the same cultivar in each pot, forming a square, as shown in Figure 1.

Treatments consisted of four combinations of weed density and distribution: 1 - without pigweed (control); 2 - four pigweed plants among lettuce plants (planting row and inter-row); 3 - four pigweed plants outside planting rows; and 4 - one pigweed plant at the center of the plot (Figure 1). We used these treatments to the four lettuce cultivars (Verônica, Lídia, Lucy Brown, and Salad Bowl). Density corresponded to 16 pigweed plants m⁻² for treatments with four weeds per pot, and 4 plants m⁻² for the treatment with one weed per pot.

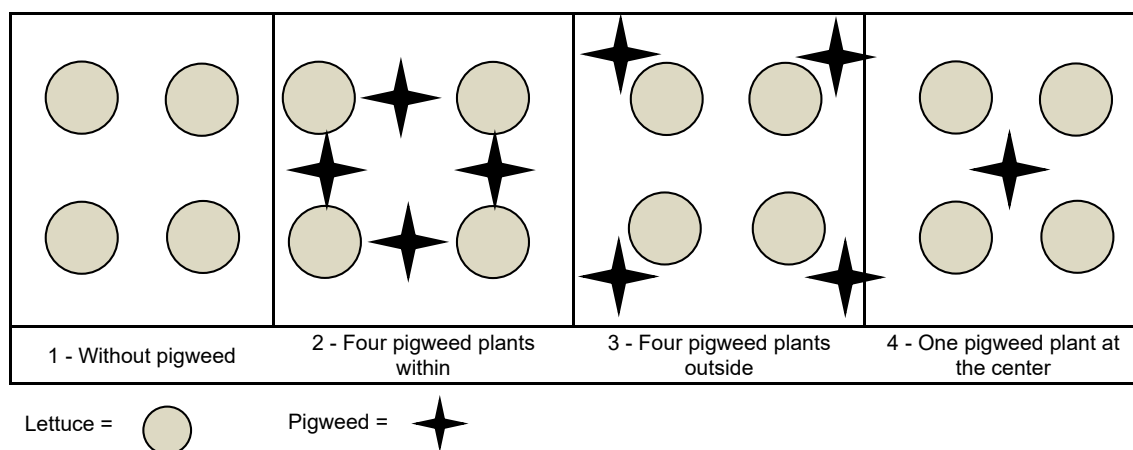


Figure 1 - Plants arrangement in the experimental plots.

A completely randomized design with three replicates was used. For pigweed evaluation, we considered treatments in which the weed coexisted with the four lettuce cultivars.

Soil fertilization occurred at 20 and 30 days after planting (DAP) of lettuce seedlings to provide nitrogen. For that, we applied a dose equivalent to 40 kg ammonium sulfate ha⁻¹ between crop rows and on the sides of the pot.

The experiment was conducted without water restriction, performing daily irrigation. To avoid the interference of other weeds in the experiment, we removed them through manual weeding whenever they emerged.

At 42 DAP, the end of the experimental period for the cultivars Lídia, Verônica and Salad Bowl; and at 52 DAP for Lucy Brown, the lettuce and pigweed plants were cut close to the soil and taken to the laboratory. The lettuce plants were washed in clean water, immediately dried with paper towels and weighed to determine the fresh matter. Then, the leaves were detached, counted and their leaf area was determined (LiCor, mod. LI 3000 A); the length of the stem was measured with a ruler graduated in millimeters. The plants leaves and stems were placed in paper bags and taken to an air-forced circulation oven at 70 °C, for 96 hours, to determine the dry matter.

For pigweed plants, the height, number of leaves, leaf area and dry matter were evaluated using the same methodology previously described.

The collected data were submitted to analysis of variance (F test), and the means compared by the Tukey test at 5% probability, using the AgroEstat software (version 1.1.0.626) (Barbosa and

Maldonado Jr., 2011). We considered a 4x4 factorial scheme for lettuce, and a 4x3 factorial scheme for pigweed.

3 RESULTS AND DISCUSSION

The factors “lettuce cultivar” and “pigweed density/distribution” interacted significantly for all the characteristics evaluated in lettuce plants.

After coexisting with four pigweed plants located outside planting rows (Table 1A), the number of leaves of cultivar Lídia decreased by 36% (Table 1A) compared to the control. For the same cultivar, this treatment did not differ from that with four pigweed plants within crop rows and inter-rows.

In the absence of competition, comparison between lettuce cultivars showed the least number of leaves for cultivar Verônica (Table 1A). When coexisting with pigweed plants, regardless of density and distribution, Verônica again showed lower values for this variable compared to the other cultivars (Table 1A).

Regarding lettuce fresh matter (Table 1B), Salad Bowl showed lower values when living with four weeds located within planting rows and with one weed at the center of the plot. This variable decreased by 61% on average for cultivar Lídia when cultivated with four pigweed plants, regardless of their location. Cultivar Verônica showed higher fresh matter for plants grown without competition, differing from the other treatments. In turn, this variable did not decrease for cultivar Lucy Brown when coexisted with one weed per plot, with values equal to the control (Table 1B).

Cultivar Lucy Brown obtained the highest values for this variable in all treatments. Cultivar Salad Bowl equaled these values only when cultivated with four pigweed plants planted outside crop rows (Table 1B).

Table 1 - Effect of coexistence between lettuce cultivars and pigweed plants on the evaluated characteristics of lettuce plants at the end of each growing cycle

Density/distribution	A) – Number of leaves				
	Lucy B.	Salad B.	Lídia	Verônica	F
Control	22.8 Ab	20.9 Ab	28.6 Aa	16.9 Ac	25.9**
4 Within	19.5 Aa	19.5 Aa	21.8 BCa	14.2 Ab	11.4**
4 Outside	20.7 Aa	21.4 Aa	18.3 Ca	13.9 Ab	12.5**
1 Central	23.1 Aa	18.8 Ab	25.3 ABa	15.1 Ac	22.7**
F	3.26 ^{ns}	1.63 ^{ns}	21.5**	1.98 ^{ns}	---
	B) – Fresh matter (g)				
Control	208.5 Aa	130.2 Ab	129.9 Ab	117.1 Ab	29.3**
4 Within	128.8 Ba	74.1 Bb	46.4 Cb	74.1 Bb	19.9**
4 Outside	137.7 Ba	123.5 Aa	55.2 Cb	67.7 Bb	27.7**
1 Central	212.9 Aa	86.4 Bb	98.8 Bb	80.4 Bb	65.7**
F	33.7**	12.7**	25.4**	8.17**	---
	C) – Leaf area (cm ²)				
Control	2772.7 Aa	2261.4 Aa	2283.6 Aa	1692.1 Ab	10.4**
4 Within	2112.4 Ca	1248.8 Bb	1107.5 Bb	1071.2 Bb	12.9**
4 Outside	2236.1 BCa	1898.2 Aa	1091.4 Bb	998.2 Bb	19.7**
1 Central	2685.9 ABa	1305.3 Bb	2441.3 Aa	1329.4 ABb	28.3**
F	5.70**	12.7**	28.7**	5.26**	---
	D) – Plant height (cm)				
Control	4.30 Cb	6.01 BCa	4.31 ABb	4.17 ABb	4.98**
4 Within	6.40 Aa	7.01 ABa	3.28 ABb	4.77 Ab	18.4**
4 Outside	4.87 BCb	8.36 Aa	3.04 Bc	2.94 Bc	41.7**
1 Central	5.94 ABa	5.40 Cab	4.59 Aab	4.19 ABb	4.04*
F	6.00**	10.9**	3.74*	3.85*	---
	E) – Total dry matter (g)				
Control	8.70 Aa	5.88 Ab	5.15 Ab	5.12 Ab	22.9**
4 Within	6.32 Ba	3.49 Cb	2.81 Cb	3.54 Bb	19.2**
4 Outside	7.06 Ba	5.39 ABb	3.27 BCc	3.79 ABc	23.4**
1 Central	8.72 Aa	4.04 BCb	4.39 ABb	3.84 ABb	43.1**
F	11.6**	10.1**	9.02**	4.04*	---

Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ by the Tukey test at 5% probability. * and ** = significant at 5% and 1% probability by the Tukey test, respectively. ^{ns} = not significant at 5% probability.

Moreover, cultivar Lucy Brown showed the highest leaf area (Table 1C) in the treatment with four pigweed plants within planting rows, differing from the other cultivars. Compared to the control, leaf area production was on average 30% lower for cultivar Verônica (Table 1C).

Considering pigweed interference with lettuce, cultivars Lídia and Verônica showed the same pattern of behavior, in which the control had the highest values for leaf area, not differing from the treatment with one pigweed per plot (Table 1C).

For plant height (Table 1D), Salad Bowl invested more in stem growth when living with four weeds. For Lucy Brown, the treatment without competition showed the lowest values, not differing from the treatment with four pigweed plants outside planting rows (Table 1D).

Comparing the cultivars in each condition of pigweed coexistence, Salad Bowl had the highest stem length, surpassing all other cultivars in the control treatment and treatment with four pigweed

plants outside planting rows (Table 1D). In the competition with four pigweed plants within planting rows and with one pigweed plant at the center of the plot, Lucy Brown equaled Salad Bowl with the highest stem length, differing from the other cultivars (Table 1D).

Regarding total dry matter (TDM) of lettuce plants, cultivar Lucy Brown showed the highest values in all conditions tested, differing from the others (Table 1E).

Comparing the dry matter production of each lettuce cultivar separately, the treatment that most affected plants was that with pigweed plants within planting rows (Table 1E and Figure 2). In this condition, cultivar Lídia had more yield losses, with a reduction of approximately 45% in dry matter compared to the control, followed by Salad Bowl, with 41%; Verônica, with 33%; and Lucy Brown, with 28% (Figure 2).

In general, considering all treatments with pigweed plants within the plot (regardless of density/distribution), cultivar Lídia was the most affected by

interference, with an average yield loss of 32.3% (Figure 2). Conversely, Lucy Brown proved to be the most tolerant to living with this infesting species, since its yield loss averaged was 6.16% (Figure 2).

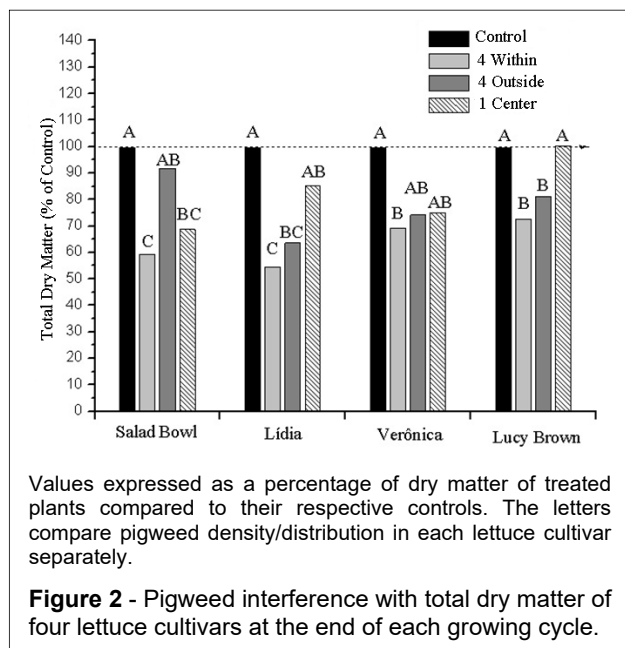


Figure 2 - Pigweed interference with total dry matter of four lettuce cultivars at the end of each growing cycle.

With the exception of stem dry matter, the treatment with one pigweed per plot obtained the highest values for all variables measured in the weed, regardless of the lettuce cultivar with which it coexisted (Table 2). As for the effects of lettuce cultivars, the plants that coexisted with Lucy Brown showed the highest height and number of leaves (Table 2).

Pigweed plants that coexisted with cultivar Lucy Brown showed higher stem dry matter (Table 3), regardless of weed density/distribution. This is probably because they remained planted for another ten days compared to the others, due to the longer

cycle of this cultivar. However, when there was only one pigweed per plot, Salad Bowl was the cultivar that most interfered with weed growth, leading to lower dry matter compared to the other cultivars (Table 3). In view of this interference, there was no difference in the pigweed growth related to the tested densities/distributions for Salad Bowl. Conversely, for cultivars Lídia, Verônica, and Lucy Brown, pigweed plants grown in the center of the plots showed higher dry matter, differing from the treatments with higher weed density (Table 3).

The weed interference in the evaluated characteristics of lettuce cultivars, such as plant height, fresh matter, leaf area, and total dry matter (Table 1B to E), corroborates studies on several cultivars (Shrefler et al., 1994; Silva et al., 1999; Machado et al., 2009; Giancotti et al., 2010; Otero and Wright, 2013). The decrease in plant growth is due to the fact that the first weeks after lettuce planting are the period in which the seedlings are susceptible to weed competition, causing yield losses of up to 85% (Blanco, 1983; Otero and Wright, 2013).

The reason for this yield loss is that weeds compete with the crop for water, light, and nutrients, which can have affected some physiological characteristics of lettuce, such as the production of xanthophylls and β -carotene, as well as chlorophyll content, thereby reducing its growth (Giannopolitis et al., 1989; Parry and Shrestha, 2018).

For the different characteristics evaluated in the lettuce plants, it was possible to observe that the cultivars responded differently to the imposed treatments, as occurred for the fresh matter, leaf area and total dry mass (Table 1B, C and Figure 2). This

Table 2 - Effect of coexistence between lettuce cultivars and pigweed plants on number of leaves (No. leaves), leaf area (LA - cm²), plant height (cm), leaf dry matter (Leaf DM - g), and stem dry matter (Stem DM - g) of pigweed plants at the end of the experimental period

Factor	Variable				
	No. leaves	LA (cm ²)	Plant height (cm)	Leaf DM (g)	Stem DM (g)
Lettuce cultivar (L)					
Lucy B.	207.5 A	1674.5 A	94.7 A	9.36 A	20.1
Salad B.	156.1 B	1450.7 AB	58.3 B	7.25 B	6.07
Lídia	141.5 B	1512.8 AB	59.3 B	8.12 AB	7.68
Verônica	165.7 B	1351.5 B	56.7 B	8.29 AB	7.37
Pigweed density/distribution (P)					
4 Within	126.5 B	1062.6 B	64.4 B	5.29 B	6.91
4 Outside	126.7 B	985.4 B	63.2 B	5.60 B	8.32
1 Central	249.9 A	2444.2 A	74.1 A	12.8 A	15.7
F Lettuce	11.1**	4.26*	74.4**	3.23*	162.3**
F Pigweed	93.9**	208.2**	910.5**	110.2**	111.2**
F LxP	2.08 ^{ns}	0.17 ^{ns}	0.56 ^{ns}	0.88 ^{ns}	18.8**
CV (%)	15.2	13.1	9.47	17.5	15.1

Means followed by the same letter in the column do not differ by the Tukey test at 5% probability. * and ** = significant at 5% and 1% probability by the Tukey test, respectively. ^{ns} = not significant at 5% probability. CV = coefficient of variation.

Table 3 - Effect of the interaction between lettuce cultivar and pigweed density/distribution on pigweed stem dry matter (g)

Cultivar	Density/distribution			F
	4 Within	4 Outside	1 Central	
Lucy B.	12.8 Ac	16.1 Ab	31.5 Aa	125.4**
Salad B.	5.07 Ba	6.31 Ba	6.84 Ca	1.02 ^{ns}
Lídia	5.14 Bb	5.60 Bb	12.3 Ba	20.1**
Verônica	4.62 Bb	5.41 Bb	12.1 Ba	21.1**
F	19.4**	33.1**	147.5**	---

Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ by the Tukey test at 5% probability. * and ** = significant at 5% and 1% probability by the Tukey test, respectively. ^{ns} = not significant at 5% probability.

distinction in response is due to the fact that several factors can differently affect the degree of weed interference in the crop (Bleasdale, 1960; Pitelli, 1985), such as: weed species, density, and distribution; edaphoclimatic characteristics of the region; period and time of coexistence between the weed and the crop; and the species or cultivar evaluated. Thus, it was possible to observe that Lucy Brown was more tolerant of coexisting with pigweed in the density of 4 plants m⁻² (one pigweed per plot), while Salad Bowl was the most sensitive to this coexistence condition (Figure 2). Conversely, Salad Bowl was the only cultivar that was able to interfere in the pigweed growth in the density of 4 plants m⁻², in order to make it equal to the dry matter produced in the plots with 16 plants m⁻² (Table 3).

Brandão et al. (2016) evaluated the weed suppression potential of several lettuce cultivars in Seropédica city, Rio de Janeiro State. The authors emphasized that the different development behaviors of the cultivars led to weed suppression due, among other factors, to soil cover. Thus, as stated by Pitelli and Durigan (1992), it is possible to observe that, within the same species, different cultivars have different capacities to support and deal with the interference imposed by weeds.

Regarding productivity, in the present study, the treatment that caused the greatest interference in the growth of all lettuce cultivars was that which consisted of four pigweed plants per plot, positioned within planting rows (Figure 2). In this coexistence condition, Lucy Brown was the cultivar with the lowest yield losses compared to the control, averaging 27.4%, followed by Verônica (30.9%), Salad Bowl (40.6%), and Lydia (45.4%) (Figure 2).

Weed density and distribution are important factors in the level of interference with the crop (Bleasdale, 1960; Pitelli, 1985). In general, it is expected that the greater the density of plants competing for environmental resources, the lower the availability of

these resources to the crop, resulting in yield loss (Shrefler et al., 1991, 1994).

In this context, Shrefler et al. (1994) studied the coexistence between *A. spinosus* (spiny pigweed) and lettuce, and found that the density of 2 weeds m⁻² was enough to reduce the fresh matter of the crop by 24%, compared to the control, after seven weeks of competition. In the present study, the density of 4 pigweed plants m⁻² reduced the fresh matter of Salad Bowl by 33.7%, while the density of 16 pigweed plants m⁻² reduced this variable by 64.2% for cultivar Lídia, compared to their respective control (Table 1B). For an even larger infestation, Shrefler et al. (1991) reported that the presence of 120 *A. lividus* plants m⁻², for more than 40 days, led to complete loss of harvest.

For the present study, it is important to emphasize that we analyzed the weed effect on the crop individually in each lettuce plant. Thus, in the pots containing four weeds within planting rows, it is possible to infer that each lettuce plant was directly affected by two weed plants (scheme shown in Figure 1). In the pots containing four pigweed plants outside planting rows, each lettuce plant coexisted directly with one weed plant. On the other hand, with one pigweed per plot, each lettuce plant was affected by “1/4” of weed. Hence, the highest competition for space occurred in the coexistence of cultivars with four pigweed plants within planting rows (density of 16 plants m⁻²), followed by four pigweed plants outside planting rows and, finally, one pigweed plant (4 plants m⁻²). When comparing the treatments with 16 pigweed plants m⁻², although the density and resources in the environment are the same, the weed position was a determining factor for the different levels of interference with lettuce plants. This difference occurred for the evaluated characteristics of the different cultivars (Table 1B to E) and corroborate Bleasdale (1960) and Pitelli (1985), who emphasized that weed position is a decisive factor for the level of weed interference with crops.

Coexistence with pigweed plants negatively affected several characteristics of the lettuce cultivar Salad Bowl (Table 1B, C and E). However, when competing with four weeds located outside planting rows, its plant height increased by 39% compared to the control (Table 1D). When compared to the other cultivars, this was the only one that maintained fresh matter and leaf area values similar to the control, when coexisting with four weeds located outside planting rows (Table 1B and C). This indicates a different behavior of the cultivars regarding shading.

Pitelli and Durigan (1992) emphasize that competition for light is an important factor to be avoided, especially in low-height crops, and, therefore, weeds must be removed before the shading becomes effective.

Etiolation is a process in which plants invest in height growth to avoid competition for light. In this situation, the auxins that would be translocated to the root via a central route in the stem are moved to an external route. This increases auxin levels in the leaves and outermost cells of the stem, which decreases leaf expansion and increases stem cell elongation, thereby increasing plant height (Morelli and Ruberti, 2002).

Regarding the interference of cultivars with the growth of pigweed plants, the results are probably relate to an allelopathic effect, besides the competition for environmental resources. In this sense, it is worth mentioning the work of Chon et al. (2005), who observed an allelopathic effect of the lettuce cultivar Ddukseom on alfalfa plants. These authors found reductions of up to 70% in the alfalfa root growth and attributed this effect to the fact that lettuce plants have several water-soluble substances, which are fruits of secondary metabolism, and have an allelopathic activity. However, the authors claim that this effect may vary according to the cultivar. Thus, this may be one of the justifications for Salad Bowl to be the only one to suppress the pigweed growth, when planted in the center of the plot, in order to equal the treatments with the highest density (Table 3).

4 CONCLUSIONS

We concluded that Lucy Brown lettuce is more tolerant to coexistence with pigweed plants, while the cultivar Lídia was the most sensitive to competition. The density of 16 pigweed plants m⁻² affected more negatively the growth of lettuce cultivars, and weed position in the planting rows interfered the most with crop growth.

5 CONTRIBUTIONS

EC: investigation and writing (original draft, review and editing). ALB: data curation, formal analysis and writing (original draft, review and editing). JSR: writing (original draft, review and editing). RTSS: writing (original draft, review and editing). PLCAA: conceptualization, methodology, project administration and writing (original draft, review and editing). ABCF: methodology, project administration and writing (original draft, review and editing).

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