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Increase of Atmosphere CO_2 Concentration and Its Effects on Culture/Weed Interaction

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Abstract**—**Climate change projections based on the emission of greenhouse effect gases suggest an increase in the concentration of atmospheric carbon dioxide, in up to 750 ppm. In this scenario, we have significant changes in plant development, and consequently, in agricultural systems. This study aims to evaluate the interaction between culture (*Glycine max*) and weed (*Amaranthus viridis* and *Euphorbia heterophylla*) in two conditions of CO_2, 400 and 800 ppm. The results showed that the coexistence of culture with both weed species resulted in a mutual loss, with decrease in dry mass productivity of culture + weeds, in both conditions of CO_2. However, when the culture is grown in association with *E. heterophylla*, total dry mass of culture + weed was smaller at 800 ppm. Soybean was more aggressive in comparison to the *A. viridis* in both the concentrations of CO_2, but not in relation to the *E. heterophylla*.

Keywords**—**Plants interaction, increase of [CO_2], plants of metabolism C3, *Glycine max*.

International Science Index, Agricultural and Biosystems Engineering Vol:11, No:6, 2017 waset.org/Publication/10007219

I. Introduction

OVER the years, the carbon dioxide (CO_2) concentration on the surface of the Earth has increased from 280 ppm in the pre-industrial period to approximately 403 ppm in January 2016 [1], and will continue to increase and may reach 750 ppm by the end of the 21st century [2].

CO_2 is the essential subtract for the photosynthetic process that occurs in plants, and this increase in the atmosphere can significantly impact food production [3]. Studies with different cultures have shown that the increase of CO_2 causes many physiological changes, photosynthetic rate, and CO_2 assimilation in plants, absorption and translocation of nutrients, water use efficiency (WUE), gene expression and enzyme activity as well [4]-[6], especially when other resources such as light, water and nutrients are not limiting [7], [8].

Once the current of CO_2 (~ 380 ppm) is a limiting factor to the maximum photosynthesis, the increase of this gas can influence a greater growth and productivity of C3 species [7], [9]. On the other hand, C4 species seem to be less responsive to increased CO_2 in comparison to the C3 species [10], [11]. In this manner, it is expected that C3 species can present a competitive advantage over C4, in high CO_2 environment [12].

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Another factor that can also limit the productivity of crops is the biological conditions of the environment, such as pests and weeds that negatively impact growth and productivity.

During its development, the weeds have frequently adapted to the environmental changes, and its growth and development, which can be observed, was favored by global warming [13], [14]. According to [15], in the near future, increased CO_2 and competition for water could play in one of the most important roles in the culture-weed interaction. According to this author, it is likely that competition for limited resources decreases crop productivity [15], since weeds and crops occupy the same ecological niche [16].

Studies that seek to understand the competitive process between weed and culture are of the utmost importance. Among the existing methodologies, substitute schemes are an alternative to the understanding of the competitive process between plants.

The substitute series model allows the study of intra and interspecific competition [17], [18] and includes analysis of monocultures of species that could be investigated and the mixtures of them in which the proportions of the two studied species vary, while the total density is held constant for all treatments.

The substitute experiments provide information on the competitiveness of agricultural crops, weed suppression and the competitive hierarchy among cultivated species and weeds, and important information in the development of more efficient practices in the management of weeds [19], for future scenarios.

II. Material and Methods

Seeds of three species were obtained commercially, from Agrocosmos Company (specializing in commercial weed seeds) and Monsanto®. The soybean used was M7110 ipro RR, early cycle (~110 days). The seeds were sown in polystyrene trays with 128 cells, using horticultural substrate (Plantmax®). When the seedlings grow their first pair of leaves, the transplant was carried out into vases with a capacity of 7 L, filled with a mixture of soil and sand in the ratio 2:1 (v/v), used in the experiments.

A. Conduct of the Experiments

The experiments were developed in open top chambers (OTCs), installed at the College of Philosophy, Sciences and Letters, USP Ribeirão Preto, through a partnership with Prof. Dr. Carlos Alberto Martinez Y. Huaman.

The OTCs were built with metal coated plastic film

International Science Index, Agricultural and Biosystems Engineering Vol:11, No:6, 2017 waset.org/Publication/10007219

structure with dimensions of 1.5 m high by 1 m in diameter. A total of six boards were used, three of those with CO_2 concentration of approximately 400 ppm (environment treatment) and three of 800 ppm (high CO_2 treatment). In chambers with a high CO_2, maintaining the concentration around 800 ppm, a cylinder was engaged (33 kg of CO_2), through an external pipe. The injected CO_2 was mixed with natural environmental air through a fan present in the chamber, forcing air entry into the chambers (Fig. 1). Since it is a semi-open system, there was a 10% (±) variation considered for the CO_2 concentration.

Fig. 1 OTCs used in the experiments. Control without CO_2 injection (A), enriched treatment with CO_2 (B)

Irrigation was automated through a drip system triggered by a timer three times a day (Fig. 2), for a total of 350 mL/day, volume needed to keep the substrate of the field capacity.

Fig. 2 Irrigation system in the OTCs' vases

To maintain good health conditions for plants, preventive applications and curative of insecticide (Curyom® 550 EC – 150 mL ha^{-1}) and fungicides (Orkestra® SC-350 mL ha^{-1}) were applied.

B. Competition Experiment (Additive Series and Substitutes)

Initially, the weed (*Euphorbia heterophylla - wild poinsettia* and *Amaranthus viridis* - Slender Amaranth) and culture (*Glycine max*) were grown separately, i.e. no coexistence of plants, with the aim of determining the critical density of the populations (plants.m^{-2}) from which the dry matter per unit area (g.m^{-2}) becomes independent of the population, according to the "law of constant final production" [20]. We used the densities of 1, 2, 4, and 8 plants per vase, approximately equivalent to 204, 408, 816, and 1632 plants.m^{-2}. The experimental design was random blocks with subdivided portions, with three replications in factorial scheme 4 x 2, four densities (204, 408, 816, and 1632 plants m^{-2}) and two concentrations of atmospheric CO_2 (~ 400 and 800 ppm). The plots were considered the two concentrations of CO_2 and the subplots plant densities. The blocks are represented by the chambers.

50 days after the transplant, the plants were evaluated on their photosynthetic characteristics in the period of 9 to 12 hours, using the second leaf fully expanded to every weed and second trefoil for soybeans. Leaf area of plants and the dry mass of the shoot area were evaluated as well. To assess the photosynthetic characteristics, an infrared gas analyzer device (IRGA; Li-Cor 6400) was used and attached to a chamber that allows the control of light, temperature, and CO_2 concentration.

The analysis for the obtaining of the population was critical as described by [21]. With the values obtained from dry mass, the reciprocal of the biomass was calculated (1/w) by:

$$\frac{1}{W} = \frac{N}{Y} \tag{1}$$

where the reciprocal is the relationship between the density of plants (N) and biomass produced per unit area (Y). With mutual values, a linear regression analysis of model is generated:

$$\frac{1}{W} = b0 + b1 \times N \tag{2}$$

in which the value of the b stands for the biomass that an isolated plant produces and that is augmented in b1 units at each increase in the population of this specie. Maximum biomass production of the species is given by the inverse of b1:

$$Ymax = \frac{1}{b1} \tag{3}$$

The critical density of the population was adjusted by an equation of biomass produced in density function:

$$Y = \frac{N}{b0 + b1 \times N} \tag{4}$$

The critical population is a population close to the value of Y max.

As a result, two experiments were carried out in the series of substitution, considering the density of 1632 plants m^{-2}. In each series, the proportions between plants of soybeans and slender amaranth (experiment 1) and between soybeans and wild poinsettia (experiment 2) were 100:0 (pure stand of g. max), 75:25, 50:50, 25:75 and 100:0% (pure stand of slender amaranth or wild poinsettia).

50 days after the transplant, the plants were evaluated by the leaf area and dry mass of the shoot area. For soybeans, the photosynthetic characteristics, quantum efficiency of photosystem II and chlorophyll content were evaluated as well, following more criteria adopted in the series.

International Science Index, Agricultural and Biosystems Engineering Vol:11, No:6, 2017 waset.org/Publication/10007219

The data obtained were analyzed qualitatively and quantitatively by means of graphical analysis following the models proposed by [22].

For the qualitative analysis, also called conventional method to substitute experiments [23], it was necessary to calculate the relative productivity of biomass of shoot areas of each treatment and species.

The formula of the relative productivity in density function described by [24] was used to obtain the relative productivity (YR):

$$YR = \mathrm{p} \times \frac{Ymist}{\mathrm{Ymax}} \quad (5)$$

Being p the percentage density of the species concerned, ranging from 0 to 1, Ymist is the productivity of treatment in coexistence with the second specie, and Ymax is the productivity of species in monoculture obtained in the experiment.

A spreadsheet chart was created with data from the relative productivity of each species and the total relative productivity which is the sum of the capacities on the separate species. It was also placed in the theoretical equality line, which represents the relative productivity if none of the species suffered any interference, which goes from 0 to 1 for the species separately, and a line in 1, for the relative production total. When the values of a species are above the line of equality, there was an increase in the production of the same. If the values below are the same, there was decreased productivity [25].

With the points obtained, the difference as to the line of equality was calculated. Using the t-test, it was verified that the difference between the line of equality and productivity is different, a nulled hypothesis would be when the average was equal to zero (H0 = 0; H1 ≠ 0).

The relative coefficient of overpopulation (CRO) was also calculated, proposed by [22], which measures the aggressiveness of the two species, to proportion of plant in critical density adopted, using:

$$RCO = \frac{\left(\frac{DSc\ coexistence}{DSw\ coexistence}\right)}{\left(\frac{DSc\ monoculture}{DSw\ monoculture}\right)} \quad (6)$$

where DSc is the dry mass of soybeans and MSw is the weed plant dry mass (slender amaranth or wild poinsettia).

The charts and regression analyses for this experiment were carried out with the statistical software Origin 8.0.

III. Results and Discussion

The results of the experiment of monoculture, analyzed according to the final constant production, are represented in Figs. 3-5. Maximum production expected from dry mass of shoot area (Y max) for soybeans was 4,701.92 and 4,001.83 g. m^{-2}, under the conditions of 400 and 800 ppm of CO_2, respectively (Figs. 3 (A) and (B)), whereas for the slender amaranth, the Y Max was 1,365.98 and 1,333.62 g. m^{-2}, 400 and 800 ppm (Figs. 4 (A) and (B)). For the wild poinsettia, the Y Max was increased to 800 ppm (1,564.84 g. m^{-2}) compared to 400 ppm (1,056.30 g. m^{-2}) (Figs. 5 (A) and (B)). Based on Y Max theory, it is observed that only the wild poinsettia was favored with the increase of atmospheric CO_2 by elevating its expected production in approximately 32%. In contrast, for the cultivation of soybeans and slender amaranth, this CO_2 increase represented a reduction in the maximum production of approximately 15% and 2.3%, respectively.

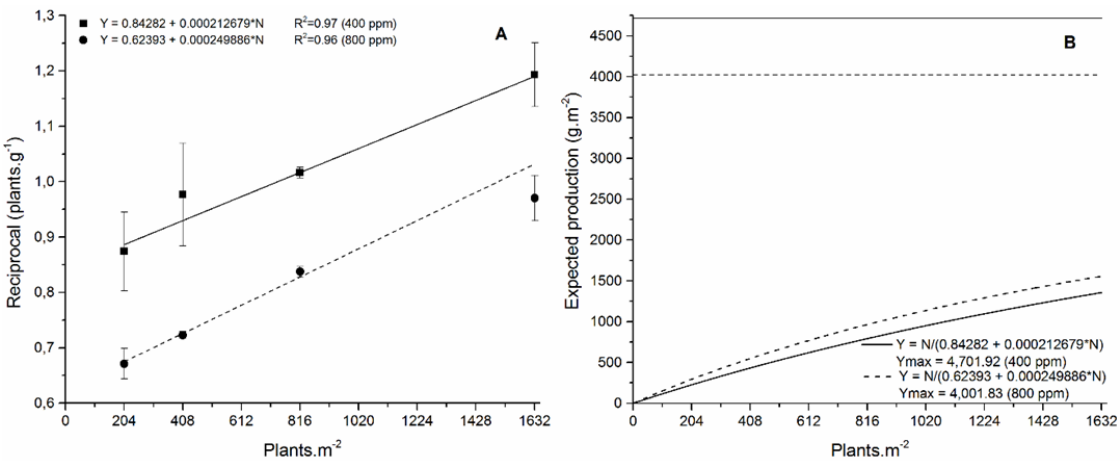

Fig. 3 Reciprocal (A) and expected production of shoot dry mass (B) of soybeans in two CO_2 conditions, 400 and 800 ppm

Based on the linear equation, it was observed that each plant increased the soy population, and the dry mass per plant was reduced by 0.00021 g when in 400 ppm of CO_2 condition and 0.00025 g in 800 ppm. The same is valid for the slender amaranth; each increased plant population, and the dry mass per plant was reduced in 0.00073 and 0.00075g, under 400, and 800 ppm, respectively. Different results were observed for the wild poinsettia, where further reduction in dry mass per plant occurred to 400 ppm (0.00095g) in comparison to 800 ppm (0.00064 g). The number of plants required for biomass, increase to be 50% of the maximum production expected (Kn) was 3,962.87 and 2,496.86 plants m^{-2} for soybeans, and 132.42

38.49 plants m^{-2} to slender amaranth, and 240.73 and 389.85 plants m^{-2} for wild poinsettia, 400 and 800 ppm of CO_2, respectively.

As more plants are required to reach 50% of the maximum production expected, i.e. the higher the Kn, the smaller the intraspecific competition. In this way, based on reduction of dry mass per plant and the value of Kn, it is evident that both soybeans and the slender amaranth presented higher intraspecific competition in condition of 800 ppm compared to 400 ppm of CO_2. In contrast, the wild poinsettia presented higher intraspecific competition of 400 ppm as compared to 800. These data can justify the fact that only the wild poinsettia has presented higher production expected to 800 ppm, since in this condition, the ability to use the resources of the natural environment for this species is greater.

The density of the plants above which there was no significantly increase of expected production was approximately 1632 plants m^{-2}, for the slender amaranth (Fig. 4 (B)). As for the soybeans and the wild poinsettia, they did not reach the maximum Y (about 9,380 and 12,000 plants m^{-2}, respectively), and a critical density of slender amaranth was used for the substitutive experiment, following the demand of this type of design in which a lower critical density must be used among the species worked.

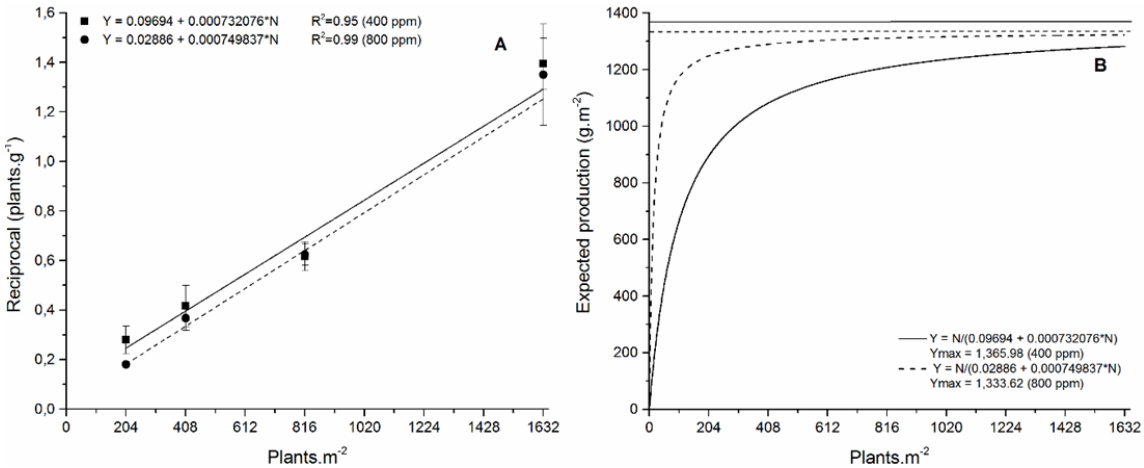

Fig. 4 Reciprocal (A) and expected production to shoot dry mass (B) of slender amaranth on two CO_2 conditions, 400 and 800 ppm

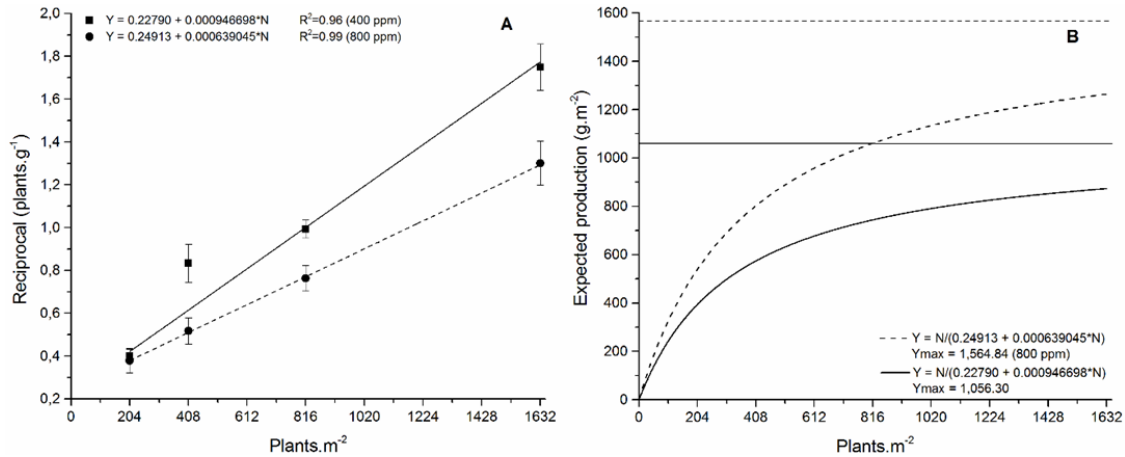

Fig. 5 Reciprocal (A) and expected production to shoot dry mass (B) of wild poinsettia in two CO_2 conditions, 400 and 800 ppm

The analyzed variable leaf area of the three species, confirms the expected production data. There was no difference in the slender amaranth leaf area between 800 ppm of CO_2 and 400 (p = 0.95869); however, there was a difference between the densities (p = 0.0019) such that the larger the number of plants m-2 increased the cumulative leaf area (Fig. 6 (B)). Note that, for this species, the cumulative leaf area tends to stabilize the higher densities, as observed with the dry mass production of the shoot (Fig. 4 (B)). For the soybeans culture, there was no difference observed in the leaf area between 400 and 800 ppm (p = 0.06314), but there was a difference between the densities (p = 4.993 * 10-13), in which the larger the number of plants m-2 increased the cumulative leaf area (Fig. 6 (A)). For the wild poinsettia, there was statistical difference to both factors studied, CO2 (p = 7.764 * 10-6), in which the leaf area was increased to 800 ppm, and density (p = 3.766 * 10-9), where the greater the number of plants m^{-2} increased the cumulative leaf area (Fig. 6 (C)). For the last two species listed, wild poinsettia and soybeans, leaf area values gained support with their dry mass production of

International Science Index, Agricultural and Biosystems Engineering Vol:11, No:6, 2017 waset.org/Publication/10007219

International Science Index, Agricultural and Biosystems Engineering Vol:11, No:6, 2017 waset.org/Publication/10007219

the shoot (Figs. 3 (B) and 5 (B)), which does not stabilize in the highest densities, or, does not reach the Y max.

The three species showed different photosynthetic characteristics (Tables I-III). There was a difference in photosynthesis (A) and WUE of the soybeans between 400 and 800 ppm of CO_2, being these two major variables in condition of 800 ppm (Table I). For the slender amaranth A, WUE and stomatal conductance (gs) were greater at 400 ppm (Table II), while for the wild poinsettia only the variable gs differed between 400 and 800 ppm, which is higher in 800 ppm CO_2 condition (Table III). There was also a difference in density function of photosynthetic plants m^{-2}, for two species of weeds. The higher the density the less the A, gs, transpiration and ICE of weeds (Tables II and III). There was no interaction between factors, CO_2 and plant density, for any of the variables in the three studied species C3 metabolism, plants like soybeans and the wild poinsettia, have better use of CO_2 through photosynthetic rate increase, when the same is found in higher concentrations in the atmosphere. This is because, under current conditions, the O_2 competes with CO_2 by the enzyme rubisco binding site, thus resulting in the loss of carbon by photorespiration. With the increase in concentration of this gas in the atmosphere, it also increases the competition of this front of the O_2 and photorespiration ceases, leading to an increase in photosynthesis, as observed in this work. The same does not tend to occur for C4 metabolism plants since under current conditions (~ 390 ppm), these plants already are at a maximum CO_2 saturation on the rubisco, because of its anatomy. In addition, as C4 plants with a smaller and more responsive CO_2, the amount of rubisco enzymes in these plants is less as compared to C3 plants, which has no positive response to the increase in this atmospheric gas.

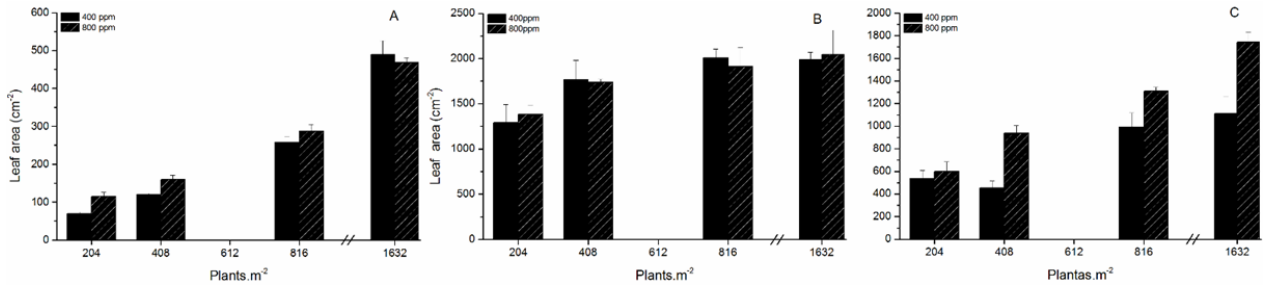

Fig. 6 Soybean leaf area (A), slender amaranth (B) and wild poinsettia (C) depending on the density of plants in two CO_2 conditions, 400 and 800 ppm

TABLE I
PHOTOSYNTHESIS (A), STOMATAL CONDUCTANCE (GS), TRANSPIRATION (E), WUE, AND INSTANT CARBOXYLATION EFFICIENCY (ICE)

	A	gs	E	WUE	ICE
		[CO_2] ppm			
400	7.76 B	0.168 A	2.25 A	3.51 B	0.024 A
800	12.24 A	0.161 A	2.14 A	5.71 A	0.019 A
		Density (plants.m^{-2})			
204	11.28 A	0.193 A	2.31 A	4.90 A	0.026 A
408	10.80 A	0.146 A	2.28 A	4.98 A	0.020 A
816	10.12 A	0.181 A	2.33 A	4.33 A	0.022 A
1632	7.81 A	0.137 A	1.86 A	4.22 A	0.018 A
		P valor			
CO_2	26.65 **	0.096 ns	0.43 ns	91.23 **	1.86 ns
Dens	3.15ns	1.77 ns	2.04 ns	2.84 ns	1.08 ns
CO_2 X Dens	0.81 ns	1.08 ns	0.03 ns	0.92 ns	0.90 ns
Normality	0.39	0.19	0.73	1	1

Averages followed by the same letter in the column with no difference among themselves, by the tukey test. * And **: significant to a 1 and 5% probability of error. NS: not significant.

Though C3 plants feature better performance with the increment of CO_2, this must be accompanied by a nutritional supply suitable; otherwise, these photosynthetic advantages may not be observed [26]. This may justify the fact that wild poinsettia did not increase photosynthetic rate at 800 ppm, as soybeans. According to [27], CO_2 levels change increases the competition between rice and *E. crusgalli* in favor of rice, since the absorption and concentration of N, P, K was higher in rice compared to the weed. The paragraph also proved to be the limiting factor for increasing rice dry mass in high CO_2 [28]. In this manner, because it is a legume with high capacity of biological fixation of nitrogen from the soil, soybeans may have been favored in relation to the wild poinsettia and thus make better use of the increment of CO_2 from the atmosphere.

TABLE II
PHOTOSYNTHESIS (A), STOMATAL CONDUCTANCE (GS), TRANSPIRATION (E), WUE, AND INSTANT CARBOXYLATION EFFICIENCY (ICE) OF SLENDER AMARANTH

	A	Gs	E	WUE	ICE
		[CO_2] ppm			
400	8.12 A	0.049 A	1.03 A	7.78 A	0.063 A
800	6.34 B	0.037 B	1.09 A	5.84 B	0.048 A
		Density (plants.m^{-2})			
204	10.76 A	0.063 A	1.52 A	7.17 A	0.081 A
408	7.56 B	0.042 B	1.07 B	7.12 A	0.066 A
816	6.04 BC	0.035 BC	0.89 B	7.00 A	0.045 AB
1632	4.56 C	0.029 C	0.77 B	5.96 A	0.03 B
		P valor			
CO_2	7.84 **	8.57 **	0.49 ns	17.70 **	2.59 ns
Dens	17.33 **	22.31 **	15.03 **	1.56 ns	6.36 **
CO_2 X Dens	0.40 ns	0.55 ns	0.87 ns	0.20 ns	0.32 ns
Normality	0.81	0.72	0.36	0.35	0.35

Averages followed by the same letter in the column with no difference among themselves, by the tukey test. * And **: significant to a 1 and 5% probability of error. NS: not significant.

TABLE III
PHOTOSYNTHESIS (A), STOMATAL CONDUCTANCE (GS), TRANSPIRATION (E), WUE, AND INSTANT CARBOXYLATION EFFICIENCY (ICE) OF WILD POINSETTIA

	A	Gs	E	WUE	ICE
		$[CO_2]$ ppm			
400	4.85 A	0.038 B	1.05 A	4.66 A	0.026 A
800	5.87 A	0.056 A	1.28 A	4.51 A	0.027 A
		Density (plants.m^{-2})			
204	8.05 A	0.058 A	1.7 A	4.69 A	0.04 A
408	5.96 AB	0.056	1.29 AB	4.65 A	0.031 AB
816	4.39 B	0.043	0.99 BC	4.29 A	0.022 B
1632	3.04 B	0.031	0.69 C	4.70 A	0.016 B
		P valor			
CO_2	1.69 ns	12.14 **	2.94 ns	0.19 ns	0.20 ns
Dens	7.51 *	5.95 **	10.53 **	0.33 ns	5.86 **
CO_2 X Dens	0.24 ns	0.26 ns	0.56 ns	1.34 ns	0.32 ns
Normality	0.86	1	0.75	1	1

Averages followed by the same letter in the column with no difference among themselves, by the tukey test. * And **: significant to a 1 and 5% probability of error. NS: not significant.

For the graphical analysis on substitution series, it is considered that if the relative production (RP) results in a straight line, there is no effect of one species over another, or the ability of the species to interfere over one another are equivalents. If the RP result is a concave line, it means damage to growth of one or both species involved; and if the RP result is a convex line, benefit the growth of one or both species. Total RP (TRP), if the same results in a straight line, being equal to 1, it means that competition occurs by the same environmental resources. Being more than a convex, there is no competition, due to the supply of resources to overcome the demand or because the species have different ecological niches; when there is less than a concave, antagonism occurs with mutual damage to the species involved [20].

The values of RP and TRP obtained in the mixture of the two species (soybeans X slender amaranth and soybeans X wild poinsettia) deviated from the expected production line at both concentrations of CO_2 (Figs. 7 and 8). In the first test, soybeans X slender amaranth, the two species produced underwhelming mass, showing a concave curve, thus demonstrating that the coexistence of these species affects the accumulation of dry mass for both species. This reduction in the RPs of species reduced also the TRP to 57 and 64% under the conditions of 400 and 800 ppm of CO_2, respectively (Figs. 7 (A) and (B)). The same happened in the second test, soybeans X wild poinsettia, in which the coexistence of species reduced the per capita resource efficiency for both. The accumulation of biomass (soybeans + wild poinsettia) was also less than 1, differed significantly between the two environmental conditions. The relative Y total presented a mass joint reduction of approximately 22% of 400 to 800 ppm (Figs. 8 (A) and (B)).

Based on polynomial equations of the series and considering a population with 10% infestation of weeds, the conviviality of culture with the slender amaranth reduces productivity to 12 and 13%, the 400 and 800 ppm of CO_2, respectively. Already in coexistence with the wild poinsettia, the soybeans had its productivity reduced to 18 (400 ppm) and 21% (800 ppm).

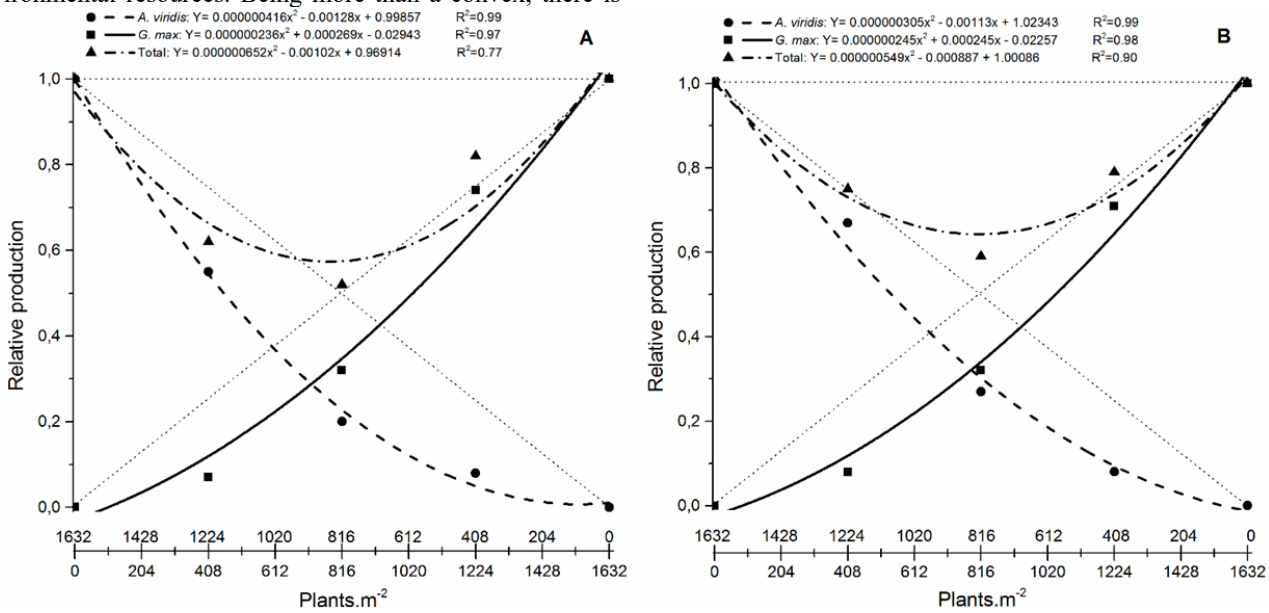

Fig. 7 Relative production of soybeans and slender amaranth to 400 ppm (A) and 800 ppm (B), substitute model

International Science Index, Agricultural and Biosystems Engineering Vol:11, No:6, 2017 waset.org/Publication/10007219

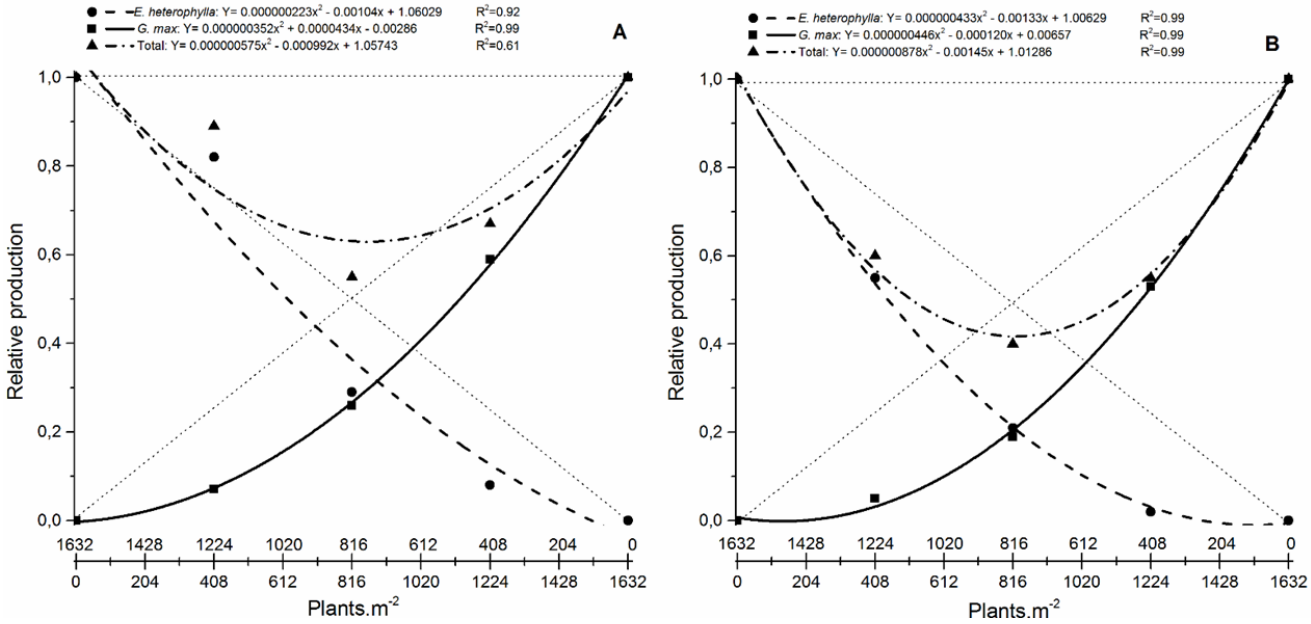

Fig. 8 Production related to soybeans and wild poinsettia to 400 ppm (A) and 800 ppm (B), substitute model

Through the analysis of the relative CRO, it was observed that when in coexistence with the slender amaranth, soy was more aggressive than the weed plant (CRO > 1) in the proportions 50:75:25 and 50 (S:SA), and only on proportion 25:75, the slender amaranth proved more aggressive than culture. When in coexistence with wild poinsettia, only in the population with the highest proportion of culture (75:25), soy proved to be more aggressive than the wild poinsettia. This occurred in a manner similar to CO_2 (Table IV). Considering a population with equal proportions of crop and weed (50:50), the density of plants studied (1632 plants m^{-2}), soybeans preferred a slender amaranth plant to a wild poinsettia. It is important to note that the anticipated changes in atmospheric CO_2 levels should not change these characteristics once in 800 ppm the wild poinsettia was even more aggressive than soybeans. However, it is observed that the increase of CO_2 in the atmosphere tends to increase the aggressiveness of soybean culture in relation to the wild poinsettia, since the CRO was 0.87 to 0.93.

TABLE IV
RELATIVE CRO OF SOYBEANS FOR THE SERIES SUBSTITUTE, SOYBEANS IN COEXISTENCE WITH SLENDER AMARANTH (SXSA) AND SOYBEANS IN COEXISTENCE WITH WILD POINSETTIA (SXWP)

	400 ppm CO_2	800 ppm CO_2
soybeans X slender amaranth		
75:25 (S:SA)	2.923	2.924
50:50 (S:SA)	1.603	1.174
25:75 (S:SA)	0.404	0.378
soybeans X wild poinsettia		
75:25 (S:WP)	2.287	7.013
50:50 (S:WP)	0.865	0.929
25:75 (S:WP)	0.250	0.272

IV. CONCLUSION

The increasing levels of CO_2 in the atmosphere impact significantly the interaction between crops and weeds in agricultural areas. That is because C3 metabolism plants can increase the demand for resources, reducing further crop productivity. In this manner, changes can occur in the weed community, modifying the weed management system.

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