Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v44i1.54392



# Growth and physiology of two sunflower cultivars fertilized with sugarcane bagasse ash

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**ABSTRACT.** One way to reduce mineral fertilizers is to use alternative fertilizers instead, such as the byproducts from the food industry. In the present study we evaluated the effects of sugarcane bagasse ash on the physiology, growth, and development of sunflowers (*Helianthus annuus* L.). We conducted an experiment in a greenhouse using a completely randomized design with two sunflower cultivars (Multissol and Catissol), five sugarcane bagasse ash doses (0.0, 3.125, 4.687, 6.25, and 7.812 mg ha$^{-1}$), and 5 replicates. At 85 days after planting we determined the plant height; leaf number; stem diameter; internal and external diameter of the flower chapter; leaf area index; shoot dry weight; net assimilation rate; dry mass production rate; relative and absolute growth rate; extravasation of electrolytes; relative water content; photosynthetic pigments (chlorophyll a, b, carotenoids, and the chlorophyll a/b ratio); and soluble carbohydrates, proteins, and proline. The height, number of leaves, and shoot dry mass increased due to the availability of nutrients contained in the ash. The incorporation of ash into the soil increased the photosynthetic activity (chlorophyll a and b) of both sunflower cultivars. The diameter of the stem, leaf area index, and relative growth rate of both sunflower cultivars increased with increasing ash dose. Therefore, the ash can be used as an alternative fertilizer, complementing or replacing mineral fertilizers.

**Keywords:** agro-industrial waste; fertilizer; *Helianthus annuus* L.; oilseed.

Received on June 20, 2020.
Accepted on November 6, 2020.

## Introduction

In Brazil, the cultivated area of sunflower (*Helianthus annuus* L.) in 2018/2019 was 62.8 thousand hectares, with a production of 104.9 million tons or 1,669 kg ha$^{-1}$. The states with the highest production were Mato Grosso and Goiás (Companhia Nacional de Abastecimento [Conab], 2020). Sunflowers are used in the food industry, primarily for their oils. Moreover, they are also used in animal feed in the form of meal or silage (Soares et al., 2016), being a culture of great international expression.

Successful sunflower crop establishment requires the use of genotypes adapted to the region, with appropriate characteristics for the place being cultivated (Dalchiavon et al., 2016). One factor that has limited the development of crops in the Northeast of Brazil is the availability of nutrients, particularly phosphorus (P), which is one of the most limiting nutrients in tropical soils (Araujo, 2011).

However, the excessive use of fertilizers polluted agricultural environments, consequently altering ecosystems, negatively affecting human health and biodiversity (Martinelli, Naylor, Vitousek, & Moutinho, 2010). In Brazil, the conciliation between modern agriculture and environmental conservation is a critical issue concerning researchers in the ecological sciences (Ferreira et al., 2012), one of the major problems is the overuse of mineral fertilizers. The fertilizers currently in use contain toxic subcomponents, such as heavy metals, inorganic acids, and organic pollutants. Therefore, over the years, due to the successive applications of nitrogen, potassium and phosphate fertilizers, these subcomponents can be accumulated, causing problems in the environment (Li & Wu, 2008).

One way to reduce the use of mineral fertilizers is to use alternative fertilizers instead. Alternative fertilizers are generally rich in organic matter composed mainly of nitrogen and phosphorus. Unlike mineral fertilizers, alternative fertilizers release nutrients throughout the crop cycle, as they are dependent on mineralization (Gliessman, 2000). Additionally, alternative fertilizers have low acquisition and production costs, which can increase the profitability of the producer (Piva et al., 2017). Among these products, ash is becoming popular, as it contains calcium and magnesium (Ribeiro, Amendola, Andrade, & Miranda, 2015) and is able to make other nutrients available to plants.

The application of wood ash, sugar cane, and industrial residues positively effects many crop species. For example, in lettuce, ryegrass, oats, corn, sorghum, cotton, castor sunflower, coffee and banana they increased plant height, diameter, number of leaves, aboveground and belowground dry mass, and leaf content of chlorophyll and nutrients (Arruda et al., 2016). Moreover, ash obtained from sugarcane bagasse was found to supply the needs of a corn crop (Feitosa, Maltoni, & Silva, 2009). Bagasse ash was also found to improve the growth of watermelon plants, increasing plant height, stem diameter, number of leaves, and leaf area (Costa et al., 2018).

However, the effects of using ash on the growth and development of crops are not yet clear. Therefore, it is important to research alternative fertilizers that meet the nutritional needs of sunflower crops and enable greater vegetative growth, maximizing productivity. Here, we aimed to evaluate the effects of different doses of sugarcane bagasse ash on the physiology, growth, and development of the sunflower cultivars Multissol and Catissol.

## Material and methods

Sunflower seeds (*Helianthus annuus* L.) of the cultivars Multissol and Catissol were provided by the Coordination of Integral Technical Assistance (Cati), and the experiment was conducted in a greenhouse at the Academic Unit of Garanhuns of the Federal Rural University of Pernambuco (UAG/UFRPE). The soil used in the experiment was collected at the UFRPE experimental farm and ash was obtained from sugarcane bagasse combustion in a bakery located in Garanhuns. Both the soil and the ash were sent to a laboratory (*Terra Análises para Agropecuária* LTDA) for chemical analysis according to the methodology described by Teixeira, Donagemma, and Teixeira (2017) and Brasil (2017), respectively. The results are shown in Table 1.

**Table 1.** Chemical characterization of soil and ash from sugarcane bagasse.

| Depth (cm) | pH | Ca | Mg | Al | H+Al | CTC | K | P | O.M | V |
|---|---|---|---|---|---|---|---|---|---|---|
| | ($CaCl_2$) | -------------------- ($cmol_c\ dm^{-3}$) -------------------- | | | | | | $mg\ dm^{-3}$ | ----------%---------- | |
| 0-20 | 6.5 | 0.8 | 0.2 | 0.4 | 2.8 | 3.95 | 0.148 | 4.0 | 2.7 | 30 |

O.M = Organic Matter; V = Base Saturation; Ca, Mg, Al, K = KCl 1 mol $L^{-1}$; H+Al = Calcium acetate pH 7.0; P = Mehlich-1; N = Kjeldahl; O.M = Muffle Dry Way.

| Sugarcane bagasse ash | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH ($CaCl_2$) | O.M | N | P | K | Ca | Mg | S | Cu | Fe | Mn | Zn |
| | ------------------------------$g\ kg^{-1}$------------------------------ | | | | | | | ---------------$mg\ kg^{-1}$------------- | | | |
| 10.4 | 87.0 | 1.0 | 11.2 | 39.6 | 107.0 | 20.0 | 12.8 | 261 | 2410 | 1200 | 239 |

Ca, Mg, K, Zn, Cu, Mn, Fe, = EDTA; S = HCl; P = Gravimetric of Quimociac; N = Raney League; O.M = Muffle Dry Way.

The soil was placed in 4 L pots and then doses of ash were added according to the treatment. We conducted an experiment in a greenhouse using a completely randomized design with two sunflower cultivars (Multissol and Catissol), five sugarcane bagasse ash doses (0.0, 3.125, 4.687, 6.25, and 7.812 mg $ha^{-1}$), and 5 replicates. Fertilization was based on the need for phosphorus (P), using ash as a nutrient source. The applied doses were 0.0 (control), 3.125, 4.677, 6.25 (recommended), and 7.812 mg $ha^{-1}$. The ash alone supplied sufficient potassium (K). To meet the crop needs for nitrogen, urea was applied at a dose of 20 kg $ha^{-1}$. Four seeds per pot were sown on September 5, 2019. Irrigation was performed daily to the point of soil friability.

Plants were evaluated at the grain filling stage, 85 days after planting (DAP). Plant height (HEI) was determined from the plant's neck to the apical meristem or inflorescence in the reproductive stage. The number of leaves (NL), that is, the photosynthetically active leaves from the apex to the neck, were determined. Stem diameter (SD) at the plant's neck was determined with a digital caliper. The internal diameter of the flower chapter (IDF, the region between the bracts) and external diameter of the flower chapter (EDF, the region composed of the floral disc) were also measured with a digital caliper. Leaf area index (LAI) was calculated using the green leaf area and the area of the pot, using the formula proposed by Floss (2004). Shoot dry weight (SDM) was determined after leaving harvested shoots in a greenhouse with forced air circulation at 65°C for 72 hours.

The net assimilation rate (NAR), dry mass production rate (DMPR), relative growth rate (RGR), and absolute growth rate (AGR) were obtained from equations proposed by Floss (2004). The extravasation of electrolytes (EE) was obtained from five leaf discs with an area of 314 $mm^2$ each, which were placed in falcon tubes containing 15 mL of deionized water. The closed tubes were stored at 25°C for 90 min. in a germination chamber, and the initial conductivity of the solution (Xi) was measured using a bench conductivity meter. Subsequently, the tubes were subjected to a temperature of 80°C for 90 min. in a water bath. After cooling the material, the final conductivity was measured (Xf). The electrolyte leakage was expressed as a percentage of the initial conductivity in relation to the total conductivity calculated using the equation proposed by Campos and Thi (1997).

The relative water content (RWC) was determined from two fresh leaves weighed immediately after collection using the equation proposed by Larcher (2000). The photosynthetic pigments (Chlorophyll a, b, carotenoids, and ratio a/b) were determined using the methodology proposed by Hendry and Grime (1993), in which the photosynthetic pigments were extracted from 50 mg of fresh mass (FM) in 5 mL of acetone solution (80%) for a period of 72 hours. The contents of soluble carbohydrates, protein, and proline were determined from leaves collected and dried in a circulating oven at 65°C for 72 hours. Soluble carbohydrates were quantified through colorimetric analysis using the phenol-sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was determined using a 50 μL aliquot of extract in a potassium phosphate buffer (pH 7.0, 0.2 M) following the method of Bradford (1976), using bovine serum albumin (BSA). Proline content was obtained from an extraction with a 5% sulfosalicylic acid solution and quantified following the method of Bates, Waldren, and Teare (1973). The data were examined using analyses of variance and, subsequently, using linear and quadratic polynomial regressions with the statistical program Sisvar (Ferreira, 2011).

## Results and discussion

Figure 1 shows the results for plant height (HEI; A), stem diameter (SD; B), leaf number (LN; C), shoot dry weight (SDM; D), internal diameter of the flower chapter (IDF; E), and external diameter of the flower chapter (EDF; F). HEI (Multissol plants only), LN, and SDM increased linearly with ash dose. In Multissol plants, HEI increased by 1.40 cm per mg of ash, LN increased by 0.6 leaves per mg of ash, and SDM increased by 0.91 g $plant^{-1}$ per mg of ash. In Catissol plants, LN increased by 0.4 leaves per mg of ash and SDM increased by 1.76 g $plant^{-1}$ per mg of ash.

In Catissol plants, SD (1B) followed a quadratic model, with the largest diameter (8.17 cm) observed at 6.59 mg $ha^{-1}$ of ash. The IDF of Multissol plants and EDF of both Catissol and Multissol plants (1E, F) were fitted to a quadratic model. The greatest IDF was 7.33 cm at a dose of 6.67 mg $ha^{-1}$ for Catissol plants. The greatest EDF was 8.41 cm at a dose of 7.55 mg $ha^{-1}$ for Multissol plants, while for Catissol plants the greatest EDF was 9.07 cm at a dose of 6.89 mg $ha^{-1}$. These plant variables are important as they can be used to predict productivity or select desirable characteristics. For example, Souza et al. (2014) found that a decrease in the diameter of the chapter had a direct effect on the potential number of achenes and reduced crop productivity in sunflowers.

We verified the beneficial effects of sugarcane bagasse ash, showing that it increased the growth of sunflower cultivars (Figure 1B, C, D, E, and F). Vegetable ash is known to improve the fertility and the physical and chemical attributes of soil (Feitosa et al., 2009). Bagasse ash is an important source of Ca and mg oxides (CaO and MgO; Table 1). It also has the ability to reduce $Al^{3+}$ levels in the soil, thus increasing the availability of nutrients for plants, as it provides carbonates (Ca and Mg) to the soil and promotes acid neutralization (Maeda et al., 2007). According to Malavolta (1989), in addition to correcting soil pH, ash contains a large amount of potassium oxides ($K_2O$) and carbonates ($K_2CO_3$), and is a source of cations.

The positive effect of ash on the availability of cations was verified by Silva, Albuquerque, Gatiboni, and Costa (2013), when applying ash from forest biomass in a humic cambisol. An increase in $Ca^{+2}$, $Mg^{+2}$ and $K^+$ cations was observed in the soil, an increase in P levels and an effective cation exchange capacity (CEC). The positive growth response of sunflower cultivars observed in our study in response to bagasse ash is likely due to increased fertility and increased saturation by soil bases (Catissol and Multissol). Ca, Mg, and K are essential macronutrients for plant growth and development. They participate in several functions in the plant, such as protein synthesis, and act as enzyme co-factors and structural components (Taiz, Zeiger, Møller, & Murphy, 2017).

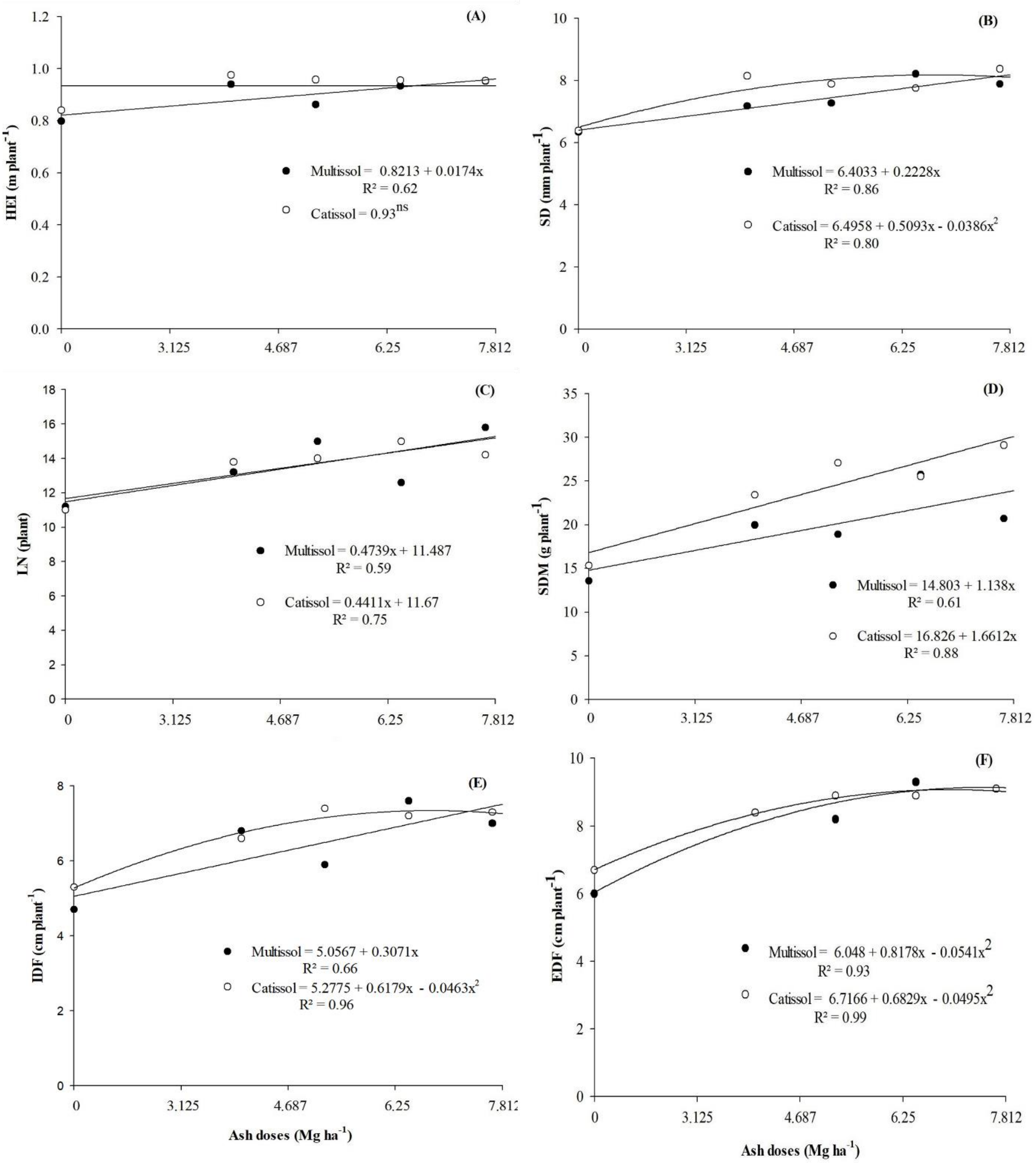


**Figure 1.** Plant height (HEI; A), stem diameter (SD; B), leaves number (LN; C), shoot dry weight (SDM; D), internal diameter of the flower chapter (IDF; E), and external diameter of the flower chapter (EDF; F) for two sunflower cultivars (Multissol and Catissol) grown with sugarcane bagasse ash doses at 85 DAP. *ns = not significant.

The ability of ash to provide nutrients and increase crop growth, as seen in Figure 1, make it a potential alternative fertilizer. Similar results have been observed worldwide. For example, Costa et al. (2018), found that sugarcane bagasse ash added at 9% of the total soil volume increased height, stem diameter, number of leaves, leaf area of plants, and gas exchange in watermelons. Similarly, Arruda et al. (2016) found that vegetable ash increased plant height, diameter, number of leaves, dry mass of shoots and roots, and leaf chlorophyll and nutrient contents in lettuce, ryegrass, oats, corn, sorghum, cotton, castor sunflower, coffee, and banana. In summary, using bagasse ash as an agricultural input allows the re-use of an industrial byproduct and improves the fertility and the physical and chemical attributes of the soil due to the amount of nutrients it contains (Feitosa et al., 2009; Ribeiro et al., 2015).

The effects of bagasse ash on extravasation of electrolytes (EE; A) and relative water content (RWC; B) at 85 DAP are shown in Figure 2. In Catissol plants, EE followed a linear trend, increasing by 5.09% at a dose of

7.81 mg $ha^{-1}$. In Multissol plants, EE did not fit either model ($p < 0.05$), and averaged 24.24%. The RWA data fitted a quadratic regression model. The lowest RWA for Catissol plants was 78.11% at a dose of 3.34 mg $ha^{-1}$, and for Multissol plants it was 80.78% at a dose of 3.46 mg $ha^{-1}$.

In Catissol plants, bagasse ash increases EE (Figure 2), suggesting that the ash resulted in a loss of cell membranes. Increases in EE are generally due to a loss of integrity or even the rupture of membranes due to an increase in the activities of reactive oxygen species (Lurie, 2003). Thus, with a loss of membrane integrity, plants tend to increase the extravasation of cells and reduce the percentage of water in their cells and organs. We found a decrease in RWC, which reflected the increase in EE up to a dose of 3.4 mg $ha^{-1}$. This reflects a physiological response in the plants; with an increase in EE there is a loss of turgor in leaves and, consequently, a reduction in RWC.

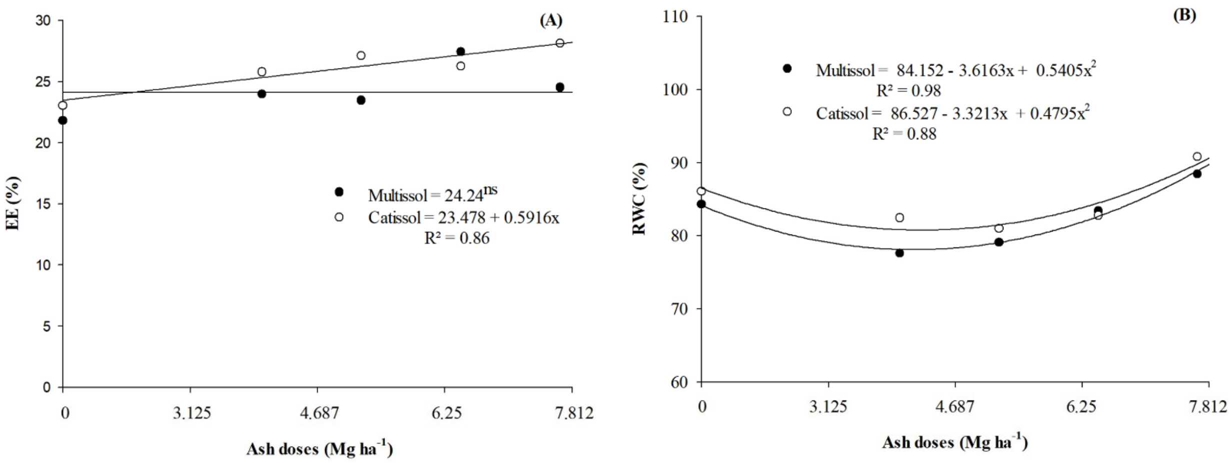


**Figure 2.** Extravasation of electrolytes (EE; A) and relative water content (RWC; B) for two sunflower cultivars (Multissol and Catissol) grown with sugarcane bagasse ash doses at 85 DAP. *ns = not significant.

Reduced water contents may reduce or completely prevent plant growth (Echer, Custódio, Hossomi, Dominato, & Machado Neto, 2010). A decrease in turgor effects several metabolic processes, including photosynthesis, since diffusion through stomata is reduced in wilted leaves (Lopes & Lima, 2015). However, in our study the increase in EE and reduction in RWC (Figure 2) were not detrimental to the growth of Catissol plants (Figure 1). However, there may have been an effect at the physiological level, as physiological symptoms often appear before morphological symptoms. Moreover, sometimes morphological symptoms do not appear because when the stress factor disappears, plant metabolism is able to return to normal functioning (Taiz et al., 2017).

Figure 3 shows the effects of bagasse ash on the leaf area index (LAI; 3A), dry mass production rate (DMPR; 3B), net assimilation rate (NAR; 3C), and absolute growth rate (AGR; 3D) of the two cultivars studied. The LAI for Catissol plants increased linearly, with an increase of 1737.40 for each mg of ash applied. In Multissol plants, LAI data fit a quadratic model, with the highest LAI (16395.54) at a dose of 5.56 mg $ha^{-1}$. In both cultivars, DMPR increased linearly, increasing at a rate of 2.54 g $m^{-2}$ $day^{-1}$ for each mg of ash applied for Catissol plants, and by 1.84 g $m^{-2}$ $day^{-1}$ for each mg of ash applied for Multissol plants.

The NAR for Catissol plants reduced linearly by $7.68 \times 10^{-4}$ g $m^{-2}$ $day^{-1}$ per mg of ash. Conversely, in Multissol plants the NAR data did not fit either of the models ($p < 0.05$), and averaged 0.0024 g $m^{-2}$ $day^{-1}$. For both cultivars, AGR increased linearly, at a rate of 0.04 g $day^{-1}$ per mg of ash applied for Catissol plants, and 0.30 g $day^{-1}$ per mg of ash for Multissol plants. The relative growth rate (RGR) data did not fit a polynomial regression model ($p < 0.05$).

The increase in LAI, DPMR, and AGR in response to bagasse ash observed in both cultivars could be related to the availability of nutrients in the ash. Application of ash has been found to increase the pH, Ca, Mg, K, and P levels in soil, and reduce the exchangeable content and potential acidity (H+Al) at doses of 0, 10, 20, 40, and 80 mg $ha^{-1}$ (Maeda, Silva, & Cardoso, 2008).

The nutrients made available by ash application result in better plant nutrition. This can increase metabolic activities (e.g. the synthesis of nucleic acids and proteins, and photosynthesis), resulting in the increase in LAI, DPMR, and AGR observed (Figure 3). Deficiencies of essential nutrients are largely responsible for reducing photosynthetic activity and reducing plant growth. In tropical regions, one of the factors that limits plant production is the availability of nutrients (Cruz, Souza Filho, & Pelacani, 2015). As ash contains Ca, Mg, K, and P (Table 1), it can play a fundamental role in plant nutrition. Wood vegetable ash has been found to increase concentrations of N, P, and K in the aboveground biomass of *Brachiaria brizantha*, and subsequently increase growth components (Bonfim-Silva, Santos, Silva, & Scaramuzza, 2014).

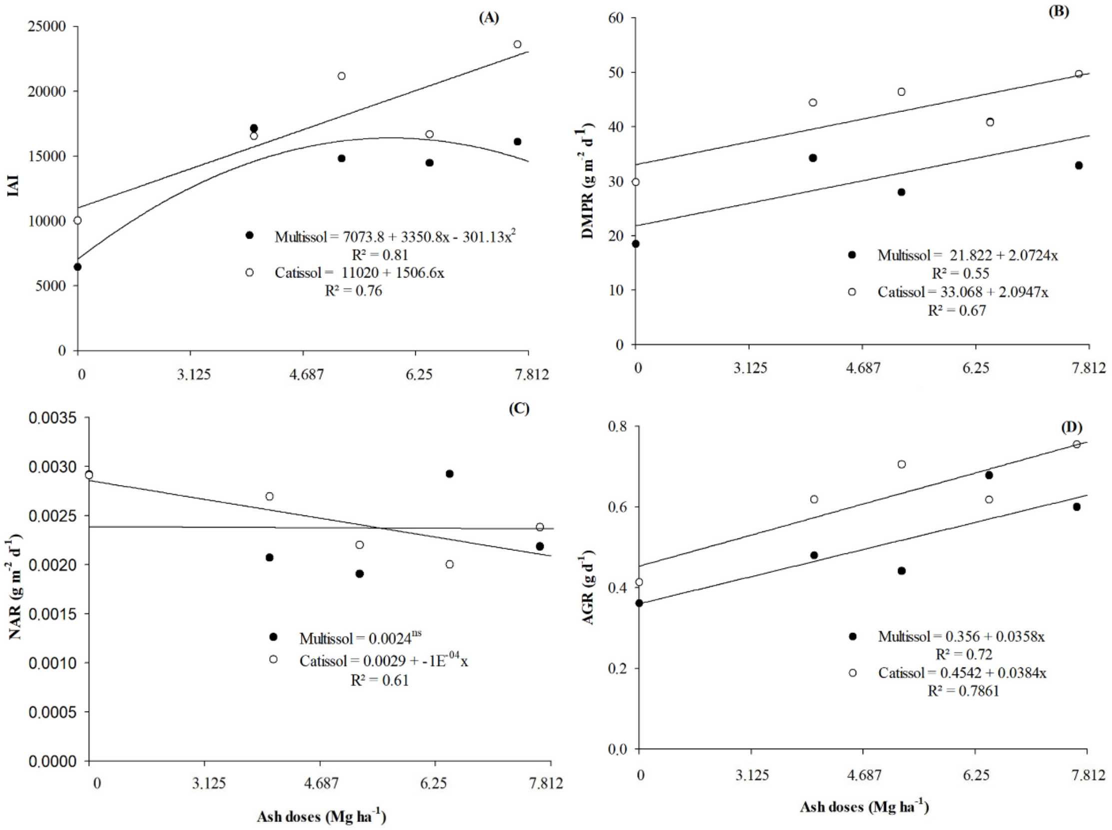


**Figure 3.** Leaf area index (LAI; A), dry mass production rate (DMPR; B), net assimilation rate (NAR; C), and absolute growth rate (AGR; D) for two sunflower cultivars (Multissol and Catissol) grown with sugarcane bagasse ash doses at 85 DAP. *ns = not significant.

Net assimilation rate (NAR) is an important variable related to growth. It describes the efficiency of net production of photosynthesis (Lopes & Lima, 2015). However, we found a decrease in NAR with increasing ash dose in Catissol plants. This could be due to the nutritional stress under the lowest doses of ash (0 and 3.124 mg $ha^{-1}$), which can alter plant cycles and cause flowering unevenness. Plants in full bloom can substantially restrict the growth of their leaves and roots, which is where a significant amount of photoassimilates are produced, for the formation, growth, and maturation of reproductive organs (Kurdali, 1996). According to Houle (2002), vegetative growth is reduced when the reproductive process starts, since the reproductive organs are the main energy drains.

The effects of bagasse ash on chlorophyll a (4A), chlorophyll b (4B), carotenoids (4C), and the chlorophyll a/b ratio (4D) are shown in Figure 4. In Multissol plants, chlorophyll a and b increased linearly, with chlorophyll a increasing at a rate of 0.08 mg $g^{-1}$ per mg of ash applied and chlorophyll b increasing at a rate of 0.025 mg $g^{-1}$ per mg of ash. For Catissol plants, the chlorophyll a and b data fit a quadratic model, with the highest concentration of chlorophyll a (1.24 mg $g^{-1}$) at a dose of 5.64 mg $ha^{-1}$, and the highest concentration of chlorophyll b (0.37 mg $g^{-1}$) at a dose of 7.34 mg $ha^{-1}$.

There were higher amounts of chlorophyll a than chlorophyll b for both cultivars (Figure 4). According to Taiz et al. (2017), this is because chlorophyll a is the main pigment involved in photosynthesis, while the other pigments (chlorophyll b and carotenoids) assist in the absorption of light and the transfer of radiant energy to the reaction centers, and are thus called pigment accessories. Mean values for chlorophyll content varied between sunflower cultivars. In studies carried out by Lee (1988), it was shown that chlorophyll content varies significantly among species and among genotypes of the same species. Photosynthetic efficiency is linked to the chlorophyll content of plants, affecting plant growth and influencing their adaptability to different environments.

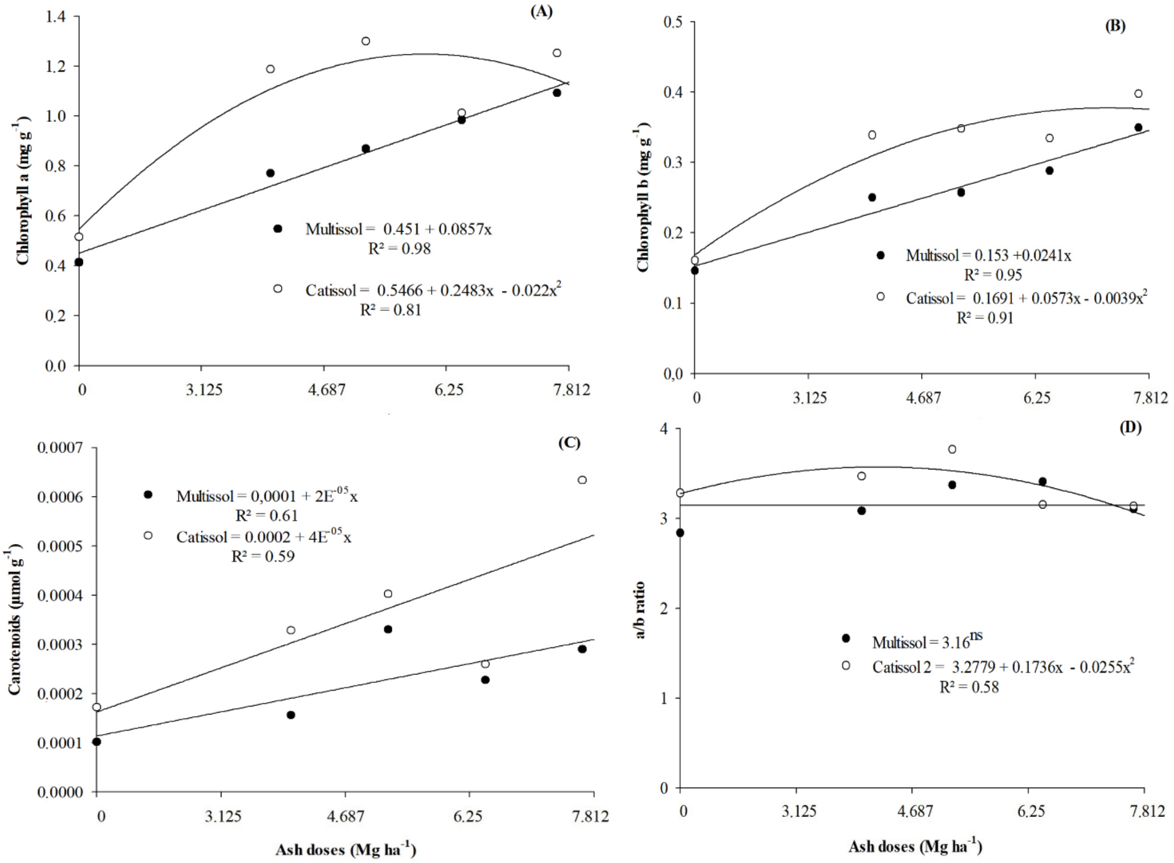


**Figure 4.** Chlorophyll a (A), chlorophyll b (B), carotenoids (C), and a/b ratio (D) for two sunflower cultivars (Multissol and Catissol) grown with sugarcane bagasse ash doses at 85 DAP. *ns = not significant.

In both cultivars, carotenoid contents increased linearly with ash dose, with increases of $5.90 \times 10^{-5}$ and $2.40 \times 10^{-5}$ μmoL $g^{-1}$ in Catissol and Multissol plants, respectively. The chlorophyll a/b ratio data for Catissol plants fit a quadratic model, with the highest ratio (4.75) at a dose of 3.40 mg $ha^{-1}$.

The levels of chlorophyll a, b, and carotenoids were influenced by the ash doses. In general, pigment contents increased with increasing doses, demonstrating the capacity of the bagasse ash to provide favorable conditions for the growth of sunflower plants. As previously discussed, ash application increases fertility due to its composition (Table 1). Among the cations contained in the ash, K and Ca may be responsible for the increased pigment concentrations. K participates in several functions in plants. It is a co-factor of more than 40 enzymes; plays a role in osmotic regulation and efficient use of water, and in the capture and synthesis of proteins; and in involved in the translocation of assimilates (Taiz et al., 2017). It also regulates the proper functioning of stomata, the transport of water and nutrients, and the thermal regulation system of plants (Nelson, Motavalli, & Nathan, 2005). Moreover, it is considered an important element in photosynthesis (Taiz et al., 2017).

An increase in chlorophyll content has been related to the availability of nutrients, since chloroplasts contain about half of the K in leaves, and K promotes greater diffusivity of $CO_2$ in mesophilic cells, thus contributing to greater photosynthetic activity (Prado & Leal, 2006). Additionally, K increases the assimilation and transport of N, and is responsible for the movement of stoma guard cells, which regulate the $CO_2$ input that serves as fuel for photosynthesis (Appezzato-da-Glória & Hayashi, 2006). Therefore, fertilization can positively influence the SPAD (Soil Plant Analysis Development) index, as long as in the fertilizer contains an adequate amount of K.

In addition to K, the ash also contained significant amounts of mg (Table 1), an essential element for the photosynthetic process. The mg could also be responsible for the increase in the amount of photosynthetic pigments observed in sunflower cultivars (Figure 4). In plants, mg activates enzymes involved in respiration, photosynthesis, and the synthesis of DNA and RNA. It is also a structural component of the chlorophyll molecule. A mg deficiency is characterized by chlorosis between the leaf veins (Taiz et al., 2017), and reduces the efficiency of the C fixation cycle, causing saturation of the electron transport system of photosynthesis and increasing the production of reactive oxygen species (ROS; Cakmak & Kirkby, 2008).

The effects of bagasse ash on carbohydrates, proteins, and proline are shown in Figure 5 A, B, and C, respectively. In the Multissol plants (Figure 5A), the carbohydrate concentration data fit a quadratic model, and the highest concentration (34.63 mg $g^{-1}$) was observed at a dose of 2.51 mg $ha^{-1}$. Conversely, the carbohydrate data for Catissol plants did not fit either model, and the average concentration was 30.83 mg $g^{-1}$. Protein concentration decreased linearly for Catissol plants (Figure 5B), reducing by 0.81 mg $g^{-1}$ per mg of ash, while for Multissol plants there was no significant effect, with protein concentration averaging 11.32 mg $g^{-1}$. For the Multissol plants, proline concentration fit a quadratic model, with the lowest concentration (4.08 mg $g^{-1}$) at a dose of 5.9 mg $ha^{-1}$. Conversely, for Catissol plants, proline concentration data did not fit either model, and averaged 5.3 mg $g^{-1}$.

An increase in carbohydrates in leaves has been related to stress in plants, as these compounds maintain leaf turgidity, even with a decrease in leaf water potential, ensuring cell expansion and growth, and can act as a signal in response to stress (Chaves, Maroco, & Pereira, 2003). In the present study, the concentration of carbohydrates was higher under smaller doses, suggesting that the absence of nutrients for plants can induce stress and the production of osmoregulatory compounds. This was also observed for the concentration of proteins and proline in the Multissol plants (Figure 5B and C); the application of ash reduced the concentration of these compounds.

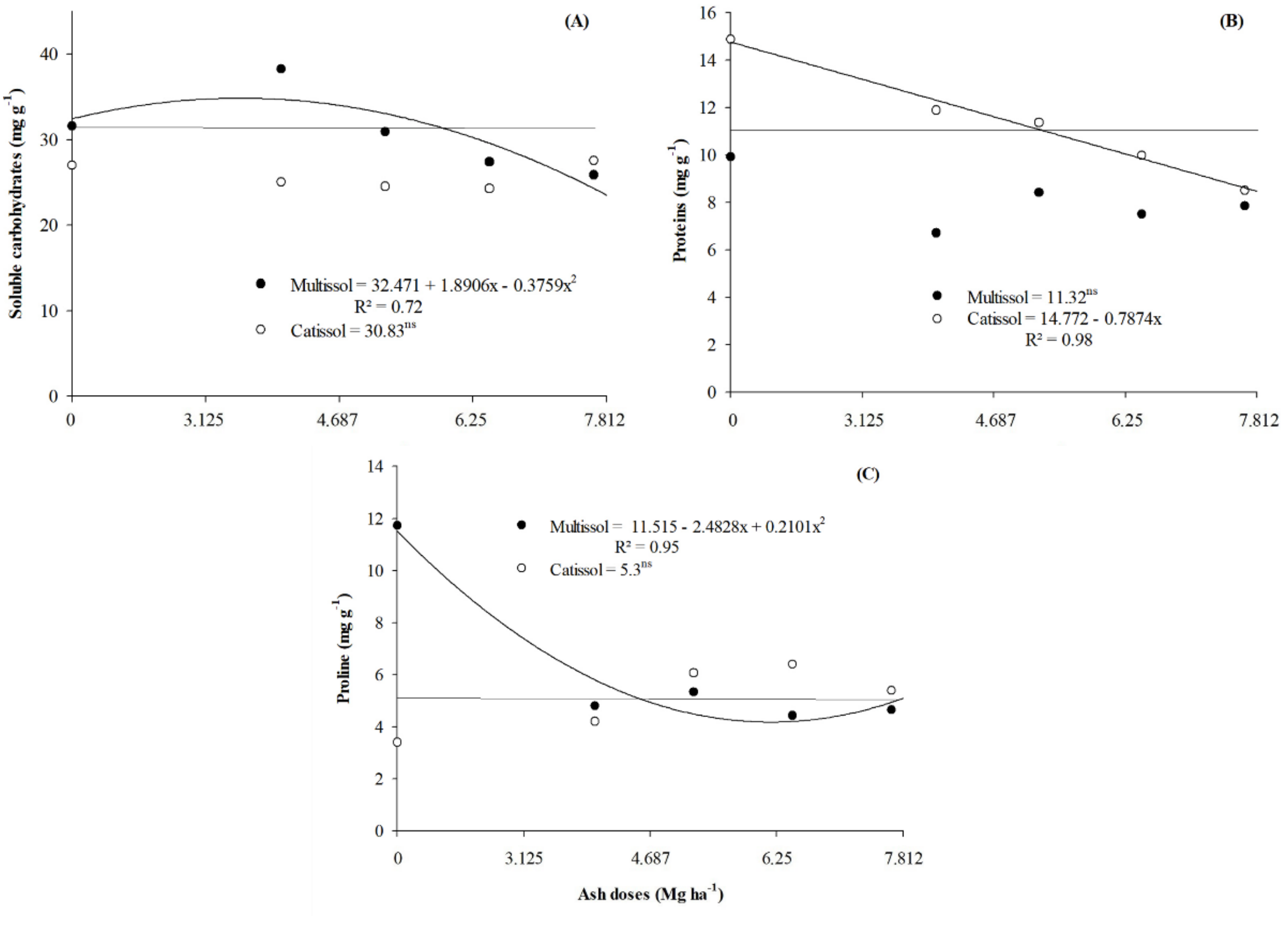


**Figure 5.** Soluble carbohydrates (A), proteins (B), and proline (C) for two sunflower cultivars (Multissol and Catissol) grown with doses of sugarcane bagasse ash at 85 DAP. *ns = not significant.

Proline is an amino acid recognized as a plant survival mechanism during stress (Teixeira, Nogueira, Maltarolo, Ataíde, & Oliveira Neto, 2015), acting as an osmotic regulator, a protector against enzymatic denaturation, a C and N reserve, and a stabilizer of protein synthesis (Delauney & Verma, 1993). Proline can also help plants maintain turgor, thus supporting cell elongation and expansion of growth regions even under stressful conditions (Premachandra, Saneoka, Fugita, & Ogata, 1992). Thus, the concentration of proteins and proline in Multissol plants was likely directly influenced by nutritional status, suggesting that it directly affects the osmoregulatory system.

## Conclusion

Fertilization with bagasse ash increased plant height, stem diameter, number of leaves, aboveground dry mass, the internal and external diameter of the chapter, the leaf area index, and the production rate of dry mass in the sunflower cultivars Catissol and Multissol. Ash also increased the photosynthetic activity of both cultivars, with the effect being more pronounced in Multissol. Sugarcane bagasse ash can be used as an alternative fertilizer to complement or replace mineral fertilizers in sunflower crops.

## Acknowledgements

We thank the Coordenation of Integral Technical Assistance (Cati), the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (Capes), and the Federal Rural University of Pernambuco (UFRPE–UAG). We would like to thank Editage (www.editage.com) for English language editing.

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