
Influence of Biotic and Abiotic Stress Factors on Physiological Traits of Sugarcane Varieties

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1. Introduction

The application of knowledge with strong physiological basis of crop yield, allied to genetic and environmental factors, is essential in developing proper practices for crop management aiming high yields. Several aspects determine the performance of a particular crop plant in a given environment, such as temperature, water availability, incidence of pests, plant genetics and management applied. Although it is virtually impossible to control all these factors, plant behavior can be assessed when submitted to different levels of these factors to understand how the responses of the plant to that given stress are formed [1; 2].

Sugarcane is the most widely planted crop in Brazil for bioenergy, being grown in about 8.1 million hectares in the year 2011 [3]. The high yields associated to the suitability for fabrication of ethanol, as well as easiness for bulk processing in high-capacity industrial facilities, catches the attention from businessmen and researchers. Furthermore, this crop is currently considered the best option for biofuel generation from the economical, energetic and environmental points of view.

There is still a big gap between physiological, high-specialized studies and application of these results for practical everyday crop management. Crop scientists, usually do not use physiological parameters in association to the directly measured variables as tools for supporting their findings. Basic research materials, which support applied studies [1; 4; 2; 5], propose changes to this scenario.

Among the factors which limit sugarcane yield, the interference caused by weed species can be highlighted. Weeds compete with sugarcane for resources as water, CO₂, nutrients, physical space and light, being responsible for considerable losses in crop yield and quality, also reducing the useful life of the sugarcane plantation under high infestation levels. The useful life of a sugarcane plantation is defined by the number of times the crop is able to sprout and form new canes, which grow from the stubble, left behind from previous harvest (called *ratoon crop*). Weed control is mandatory in sugarcane plantations, being the chemical control the most widely used method, due to its high efficiency, easiness of use and low cost compared to other control methods [6]. When plants are subjected to strong competition in the plant community, physiological traits of growth and development are usually changed. This results in differences in the use of environmental resources, especially water, which directly affects the availability of CO₂ in leaf mesophyll as well as leaf temperature, therefore, the photosynthetic efficiency. Water competition can also affect the absorption of soil nutrients by sugarcane plants.

If from one side weeds are the main biotic factor that causes reduction in sugarcane yield, herbicides used for weeds management are usually considered the main abiotic factor impacting yields. Several herbicides are usually applied at high doses in sugarcane, often causing high toxicity levels to the crop [7]. Herbicidal toxicity to sugarcane should not be evaluated based only in visual symptoms, once there are herbicides known to reduce crop yields with almost no external symptoms. There are some herbicides, however, known to cause severe external toxicity symptoms, which disappear after some days with little to no impact on final crop yield.

It is known that sugarcane varieties behave differently to some herbicides, and as consequence it is common to observe different levels of intoxication by given herbicides [7]. Thus, there is the need to classify varieties in terms of susceptibility to the most widely used herbicides for weed control in sugarcane fields. The application of herbicides will, as mandatory, cause some level of harm to crops – from almost no harm to near total plant death. These impacts are sometimes not easily visible externally at the plant, but the physiological parameters would be imbalanced resulting in lower plant performance. Because of that, more susceptible parameters like the ones associated to the photosynthesis and water use of plants are essential tools for monitoring herbicide safety for crops.

The data presented in this chapter includes the state-of-the-art about sugarcane susceptibility to herbicides, plus the most up-to-date knowledge about this subject generated by a group of researchers which are reference in herbicide physiology, from different Brazilian institutions. Parameters associated to the photosynthetic efficiency, as CO₂ concentration in the environment and in the leaf mesophyll, leaf temperature, and parameters associated to the dynamics of use of water - including Water Use Efficiency - impact the photosynthesis rate, thus affecting yield.

2. Chemical weed control

The wide acceptance of chemical weed control with herbicides can be attributed to: (1) less demand of human labor; (2) efficient even under rainy seasons; (3) efficient in controlling

weeds at the crop row with no damage to crop root system; (4) essential tool for no-till planting systems; (5) efficient in controlling vegetatively propagated weed species; and (6) allows free decision about planting system (in rows, sowing) and crop row spacing [8].

It is important to consider, however, that an herbicide is a chemical molecule that should be correctly managed to avoid human intoxication as well as environmental contamination [8]. The knowledge in plant physiology, chemical herbicide groups and technology of pesticide application is essential for the success of the chemical weed control [9]. Surely there are risks involved at this method, but if they are known they can be avoided and controlled.

Chemical weed control should be applied as an auxiliary method. Efforts should be focused on the cultural method of weed management once it allows the best conditions for the development of crops while at the same time creating barriers for the proper development of seedlings of weed species [8; 9].

3. Traits related to the photosynthesis and water use efficiency

The photosynthesis rate surely is one of the main processes responsible for high crop yields, but the liquid photosynthesis is a result of interaction among several processes, and each one of these processes, alone or in sets, may limit plant gain in terms of photoassimilates [9]. The genetic variation among species and even among biotypes of the same species may shift the enzymatic mechanism and make a given species more capable than other in extracting or using efficiently a given environmental resource, aiming to maximize its photosynthetic rate. Until recently, it was widely accepted that light affected indirectly the stomatal opening through the CO₂ assimilation dependent on light – i.e., light increased the photosynthesis rate, which would reduce the internal CO₂ concentration in the leaf and as a consequence the stomata would open [5; 10]. More accurate studies, however, concluded that stomatal response is less connected to the internal CO₂ concentration of the leaf than anticipated; most of the response to light in stomatal opening is direct, not mediated by CO₂ [10].

Distinct light regimes, both in terms of quantity and composition, influence almost all physiological processes like photosynthesis and respiration rates, affecting also variables like plant height, fresh and dry mass and water content of the plant [11]. Water content, on the other hand, shifts both the stem length and leaf area of the plant, in a way to adapt the plant to the amount or quality of light intercepted [12]. Interspecific and intraspecific plant competition affects the amount and the quality of the final product, as well as its efficiency in utilization of environmental resources [13; 14]. This is noted when assessing physiological traits associated to photosynthesis, such as concentration of internal and external gases [15], light composition and intensity [16] and mass accumulation by plants under different conditions.

Although gas exchange capability by stomata is considered a main limitation for photosynthetic CO₂ assimilation [17], it is unlikely that gas exchange will limit the photosynthesis rate when interacting with other factors. However, photosynthetic rate is directly related to the photosynthetically active radiation (composition of light), to water availability and gas

exchange. Plants have specific needs for light, predominantly in bands of red and blue [23]. When plants do not receive these wavelengths in a satisfactory manner, they need to adapt themselves in order to survive [25]. When under competition for light, the red and far-red ratio affected by shading is also important [16] and influences the photosynthetic efficiency [18].

4. Physiological parameters

Table 1 presents the main physiological parameters evaluated by the equipment called Infra Red Gas Analyzer (IRGA). Details on the principles of measurements by this equipment, as well as cares to be taken to avoid reading errors, can be found in [19], [20] and in the technical manual of the equipment. Please note that the parameters available vary among equipments from different manufacturers, as well as among models of the same manufacturer. Some of them (ΔC , T_{leaf} , ΔT and WUE) are usually not automatically supplied, but may be easily calculated based on parameters supplied by most of the equipments. The measuring units of the parameters also vary, and the most common units were adopted at Table 1. Water use efficiency (WUE) has several interpretations and may be presented in several different units, for distinct purposes. For a more comprehensive overview of this parameter, please consult [21].

Parameter	Usual Unit	Name and Description
A	$\mu\text{mol m}^{-2} \text{s}^{-1}$	<i>Photosynthesis rate</i> – Rate of incorporation of carbon molecules from the air into biomass. Supplied by equipment.
E	$\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$	<i>Transpiration</i> – Rate of water loss through stomata. Supplied by equipment.
Gs	$\text{mol m}^{-1} \text{s}^{-1}$	<i>Stomatal conductance</i> - Rate of passage of either water vapor or carbon dioxide through the stomata. Supplied by equipment.
Ci	$\mu\text{mol mol}^{-1}$	<i>Internal CO₂ concentration</i> – Concentration of CO ₂ in the leaf mesophyll. Supplied by equipment.
E _{an}	mBar	<i>Vapor pressure at sub-stomatal chamber</i> – Water pressure at the sub-stomatal zone within the leaf. Not supplied by some equipments.
ΔC	$\mu\text{mol mol}^{-1}$	<i>Carbon gradient</i> – Gradient of CO ₂ between the interior and the exterior of the leaf. Usually not supplied by equipment. May be calculated by the difference between the CO ₂ of reference (supplied by equipment) and its concentration at the mesophyll (Ci – supplied by equipment).
T _{leaf}	°C	<i>Leaf temperature</i> – Supplied by equipment in °C or °F.
ΔT	Δ °C	<i>Temperature gradient</i> – Not supplied by equipment. May be calculated by the difference between the temperature of the leaf (supplied by equipment) and the environmental temperature (supplied by equipment).
WUE	$\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$	<i>Water use efficiency</i> – Describes the relation between the rate of incorporation of CO ₂ into biomass and the amount of water lost at the

Parameter	Usual Unit	Name and Description
		same time interval. Usually not supplied. May be calculated by dividing photosynthesis (A) by transpiration (E). May be presented in several distinct units.
A/Gs	Curve*	<i>Intrinsic water use efficiency</i> – Not widely used, but describes a relation between the actual photosynthesis rate and the stomatal conductance. The original units of each parameter is maintained.
A/Ci	Curve*	<i>Photosynthesis / CO₂ relation</i> – Describes the curve of photosynthesis rate as the concentration of CO ₂ within the leaf is increased. The original units of each parameter is maintained.

Table 1. Physiological parameters usually available when using an Infra Red Gas Analyzer (IRGA). Parameters available vary among manufacturers as well as among models of the same manufacturer. The equipment supplies some parameters while others have to be calculated. * These data should be represented as a graph showing the relation between variables as the concentration of one of them is increased; thus, the original units are maintained.

5. Photosynthesis

The photosynthesis (A) and thus the respiration, depend upon a constant flux of CO₂ and O₂ in and out of the cell; this free flux is a function of the concentration of CO₂ (C_i) and O₂ at the intercellular spaces, which depend on the stomatal opening, major controller of the gas flux through stomata [22; 23]. This is mainly controlled by the turgescence both of the guard cells (which control stomatal opening) as well as by the epidermic cells at the stomata [24]. A low water potential will promote reduction in stomatal opening and reduce the leaf conductance, inhibiting photosynthesis and also the respiration [25], and increasing the gradient of CO₂ concentration between the leaf mesophyll and the exterior of the leaf (ΔC).

6. Water use efficiency

When plants are studied in terms of the efficiency they present when using water, the parameters stomatal conductance of water vapor (Gs), vapor pressure at the sub-stomatal chamber (E_{an}), transpiration rate (E) and water use efficiency (WUE) should be considered. The WUE is obtained by the relation between CO₂ incorporated in the plant and the amount of water lost by transpiration during the same period [2]. The more efficient water use is directly related to the photosynthetic efficiency as well as the dynamics of stomatal opening, because while the plant absorbs CO₂ for the photosynthesis, it also loses water to the atmosphere by transpiration, in rates that depend on the potential gradient between the interior and the exterior of the leaf [9]. Water exchange also allows the plant to keep adequate temperature levels, which can be evaluated by the leaf temperature (T_{leaf}), as well as by the difference between the leaf temperature and the temperature of the air surrounding the leaf (ΔT).

7. Interference of *Brachiaria brizantha* in the morphology and physiology of sugarcane

The study was installed at the experimental station of Horta Nova, owned by the Federal University of Viçosa (UFV), Viçosa-MG, Brazil, in an Acrisol. The planting of sugarcane was carried out under conventional system of 1-year sugarcane, after the operations of plowing and harrowing, in rows spaced in 1.4m. Planting density was 18 buds m^{-1} with fertilization performed at planting according to the results of physicochemical analysis of the soil and following the recommendations for the crop, using 500 $kg\ ha^{-1}$ NPK 08-28-16, supplemented by topdressing of 160 $kg\ ha^{-1}$ KCl.

The experimental unit measured 8.4m wide by 5.0m long (42 m^2 per plot). Treatments consisted of 12 populations of *B. brizantha*, planted among three varieties of sugarcane, at the following densities : 0, 1, 3, 7, 15, 32, 40, 32, 64, 92, 88, and 112 plants m^{-2} ; 0, 1, 4, 14, 10, 18, 28, 30, 36, 54, 52 and 72 plants m^{-2} ; 0, 1, 3, 6, 14, 20, 24, 26, 26, 32, 46 and 56 plants m^{-2} , for the varieties RB72454, RB867515 and SP801816, respectively.

The populations of *B. brizantha* were obtained by sowing 10 $kg\ ha^{-1}$ of seeds, 10 days before the emergence of sugarcane; densities for competition were established when plants were at the stage of two leaves to one tiller through thinning, carried out with application of the herbicide MSMA (Volcane® - 2 L ha^{-1}). Random plants were covered with plastic cups before application, in order to escape from the herbicide, resulting in the desired densities. Weeds other than *B. brizantha* were controlled with 2,4-D or through manual hoeing, especially the new emergences of *B. brizantha*. Herbicides were applied with a CO_2 -propelled backpack sprayer, coupled to a 2m wide bar with four spray nozzles Teejet TT 110.02, calibrated to spray 150 L ha^{-1} .

The evaluations of the final populations of *B. brizantha* in each treatment were accomplished 90 days after crop emergence (DAE), with scores in two areas of 0.25 m^2 (0.5 x 0.5 m) in each experimental plot. At 120 DAE, the stalks diameter, the number of stalks, the plant height and number of leaves per plant were measured. After these determinations, sugarcane plants were sectioned at the soil level, and stalks were separated from leaves. The leaves were placed in polystyrene boxes containing ice, to prevent dehydration, in order to determine the leaf area (LA) in electronic equipment (Licor, model LI-3100C). After LA determination, stalks were added to the corresponding leaves, and the material was placed inside ovens at 65 °C with continuous air circulation, for determination of shoot dry mass.

The yield of stalks was estimated by counting stalks present in the four central rows of 0.5m, disregarding borders on each side and front portion. Later, 30 stalks were collected at random then weighed. With the average weight of stalks and number of stalks per unit area, sugarcane yield was estimated in $t\ ha^{-1}$.

At the same time of the morphological evaluations, the physiological parameters of the treatments were evaluated by using an Infra Red Gas Analyzer (IRGA), model LCA Pro⁺. The sub-stomatal CO_2 concentration ($C_i - \mu mol\ mol^{-1}$), photosynthesis rate ($A - \mu mol\ m^{-2}\ s^{-1}$), a stomatal conductance ($G_S - mol\ m^{-1}\ s^{-1}$), transpiration rate ($E - mol\ H_2O\ m^{-2}\ s^{-1}$) water use efficiency ($EUA - mol\ CO_2\ mol\ H_2O^{-1}$) were obtained. To avoid excessive interference of the

environmental parameters on the readings, each block of the experiment was evaluated in a different day, between 07:00 and 10:00 am.

Data were tested for homoscedasticity and then submitted to analysis of variance. Subsequently, analyses of linear and nonlinear regression were performed to evaluate the effects of populations of *B. brizantha* on morphological traits of each sugarcane variety. The choice of the most suitable model for each variable was based on the statistical significance (F-test), fitting correlation coefficient (R^2) and the biological significance of the model.

The leaf area (LA) of sugarcane was reduced as the population of *B. brizantha* was increased, for varieties RB72454 and SP80-1816 (Figure 1A). Variety RB867515 was more tolerant to competition with the weed, for the same variable, because constant values of LA were observed with the increasing in the population of the competitor. Under competition, plants tend to have lower leaf area, due to the allocation of a higher proportion of dry mass in stalks, making them longer and raising the leaves in search for light. In addition, under this situation leaves usually have smaller dimensions. Similar results were found by [26] who observed that the increase in the population of ryegrass under competition with wheat, caused a decrease in leaf area but did not alter the thickness of the leaves. In some cases, under competition leaves can also become thinner, changing the leaf area while keeping constant weight. For varieties in which leaf area was not affected, it may be necessary to determine whether there were changes in leaf thickness in order to elucidate the mechanism of tolerance.

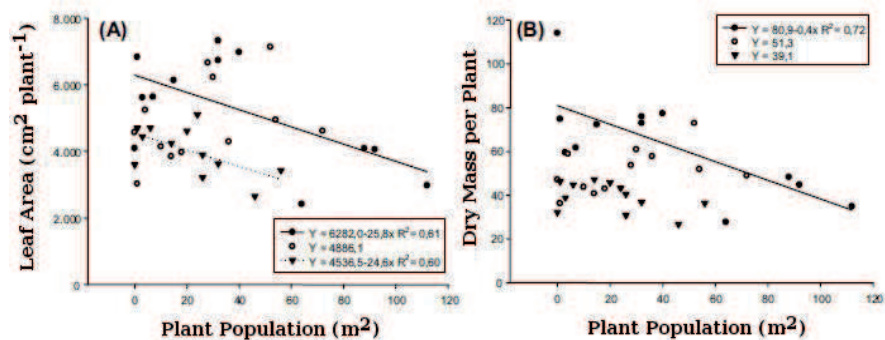


Figure 1. Leaf area (A) and shoot dry mass (B) of sugarcane varieties (●) RB72454, (○) RB758515, and (▼) SP801816 under competition with populations of *Bracharia brizantha*. DFT/UFV, Viçosa-MG-Brazil, 2008/09.

The results show that there was less accumulation of dry mass (DM) in the variety RB72454 as a function of increase in population of *B. brizantha*. Since the DM of varieties RB867515 and SP80-1816 did not change with the increasing in weed population, it may be hypothesized they are less affected by competition in the early stages of development (Figure 1B). High populations of weeds extract more resources from the environment and therefore interfere with the growth and accumulation of assimilates in crop plants, which is reflected in slower development of LA and the accumulation of plant DM [27].

With the increase in the population of *B. brizantha*, it was observed increase in stalk diameter, except for the variety RB72454 (Figure 2A). Diameter increase is generally attributed to a smaller number of tillers. The stalk diameter is directly related to the availability of resources for the crop; in case of lower resource availability, some tillers may be aborted with a possible increasing in the thickness of the remaining ones [28]. In a global context, plants are highly responsive to stress, whether natural or man-imposed, by changing their morphology towards a necessary adaptation for their survival [29].

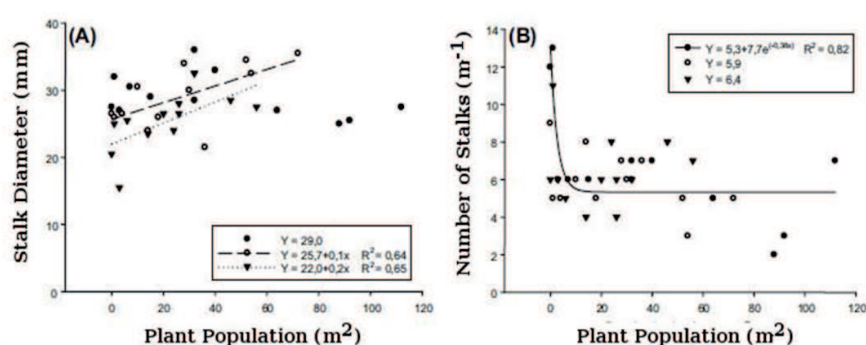


Figure 2. Stalk diameter (A) and number of stalks (B) of the sugarcane varieties (●) RB72454, (○) RB758515, and (▼) SP801816 under competition with populations of *Bracharia brizantha*. DFT/UFV, Viçosa-MG-Brazil, 2008/09.

It was also observed a reduction in the number of stalks per meter, with increases in the population of the weed for RB72454, while the other varieties were not affected (Figure 2B). This demonstrates that RB72454 is more susceptible to competition compared to the other varieties. The number of stalks of RB72454 was reduced until the population of 10 plants m⁻² of *B. brizantha* (Figure 2B).

Differences in competitive ability of crops versus weeds were observed by several researchers: sugarcane with signalgrass [30], soybean with oil radish [34], rice with barnyardgrass [31], rice with red rice [32], and sorghum with Johnsongrass [33]. Temperature, humidity, soil fertility and light are the major determinants of plant growth, each of these elements may be more or less limiting in specific situations [29].

With increases in the population of *B. brizantha*, there was an increase in plant height of varieties RB867515 and SP80-1816 (Figure 3A). The competition mainly for light, leads to the tendency of plants to invest more photosynthates in height, increasing the interception and shadowing other competing plants [35]. Competition for light between plant communities begins very early, affecting early apical dominance [36].

Some authors hypothesized that ecotypes (or varieties) of the same species might have differential capacity to adapt to distinct light intensities [37; 38]. Researches emphasize that all individuals of the same species are technically able to adjust their physiology to the light

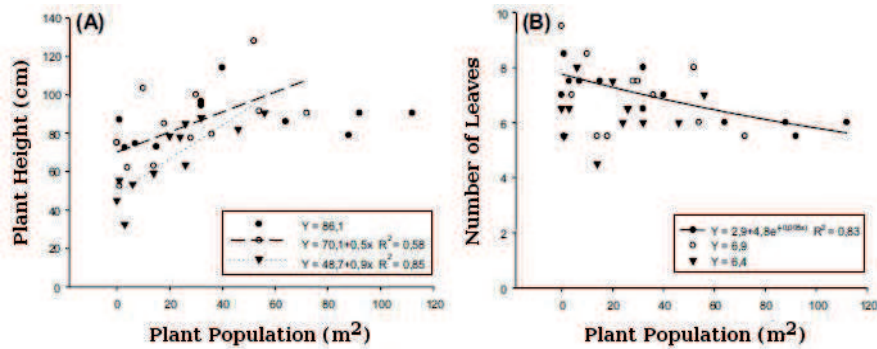


Figure 3. Plant height (A) and number of leaves per plant (B) of the sugarcane varieties (●) RB72454, (○) RB758515, and (▼) SP801816 under competition with populations of *Bracharia brizantha*. DFT/UFV, Viçosa-MG-Brazil, 2008/09.

intensity to which they are exposed, i.e., an individual which grew originally in the sun can adapt itself to a shaded environment and vice versa [39; 40]. Thus, it is implied that the varieties of sugarcane that develop simultaneously and more rapidly in height and leaf area compete more effectively for light.

The number of leaves per plant of the variety RB72454 was negatively influenced by increasing the plant population of *B. brizantha* (Figure 3B), and under these circumstances the variety RB72454 again proved to be less competitive than the others when in the presence of the competitor.

The stalk yield was reduced with the increase in the population of *B. brizantha*, stabilizing losses in populations of about 40 plants m^{-2} of the competitor, for all varieties (Figure 4). It should be noted that increased competition between sugarcane and *B. brizantha* caused reduction also in crop tillering and, consequently, fewer stems were obtained per area. According to [41] and [8], a fast and uniform initial growth is essential for obtaining a good stand, enabling the rapid closure of the canopy, which leads to better utilization of light energy and more effective suppression of weeds. Beyond 40 plants m^{-2} of the competitor the intraspecific competition between plants of *B. brizantha* could have been too strong, thus reducing the potential for competition of individual plants.

The substomatal CO_2 concentration (C_i) decreased with increasing plant population of *B. brizantha* for RB72454 (Figure 5). C_i is mainly influenced by stomatal conductance and photosynthetic activity, being considered a physiological variable influenced by many environmental factors such as water availability, light and others [9]. The variety RB72454 presented higher C_i when compared to SP80-1816 [42]. Differences in C_i among rice varieties were observed [43], and only one cultivar was influenced by the competition in this variable. Changes in the values of G_s (Figure 6) for RB72454 help explaining the lower C_i found for the same variety. With lower G_s there is a lower influx of CO_2 to the inner space of the leaf, thus, reducing the concentration of substrate (C) leading to limitations in the photosynthetic activity.

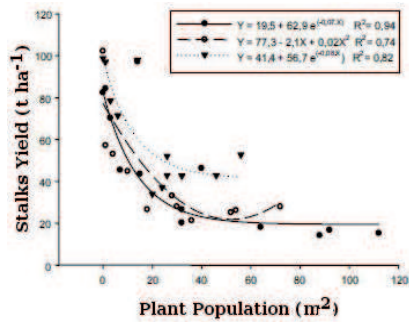


Figure 4. Stalks yield ($t\ ha^{-1}$) of the sugarcane varieties (●) RB72454, (○) RB758515, and (▼) SP801816 under competition with populations of *Brachiaria brizantha*. DFT/UFV, Viçosa-MG-Brazil, 2008/09.

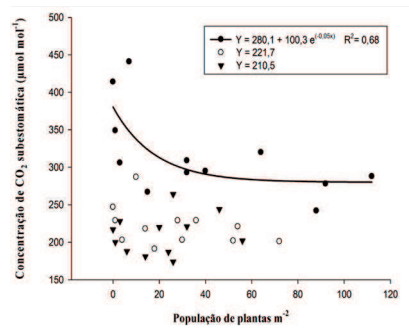


Figure 5. Substomatal CO_2 concentration ($C_i - \mu mol\ mol^{-1}$) of sugarcane varieties (●) RB72454, (○) RB857515 and (▼) SP8018-16 under competition with populations of *Brachiria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09. Figure

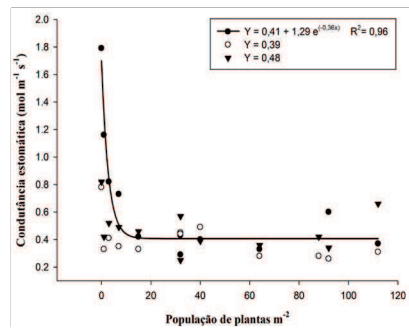


Figure 6. Stomatal conductance ($G_s - mol\ m^{-2}\ s^{-1}$) of sugarcane varieties (●) RB72454, (○) RB857515 and (▼) SP80-1816 under competition with populations of *Brachiria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09.

There was a reduction in stomatal conductance (Gs) of the sugarcane with increasing plant population of *B. brizantha* (up to 16 plants m⁻²) for the variety RB72454; the others presented stable Gs (Figure 6). Corroborating these results, there were no differences between the values of Gs among varieties in a study conducted by [42] under field conditions. Differential responses of reduced Gs in plants subjected to competition were also observed for the rice crop, where only one variety showed reduction in this variable [43].

The transpiration rate (E) for RB867515 and RB72454 was reduced as the density of plants of *B. brizantha* was increased; for the former, this reduction was observed until 40 plants m⁻² of the competitor, being stabilized under higher competition intensities (Figure 7). The reduction of E is directly related to water availability to plants [9], thus as the plant density was increased, the competition for water in the soil was also more serious.

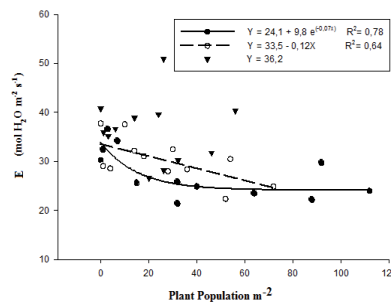


Figure 7. Transpiration rate (E - mol H₂O m⁻² s⁻¹) of sugarcane varieties (●) RB72454, (○) RB857515 and (▼) SP80-1816 under competition with populations of *Brachiria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09.

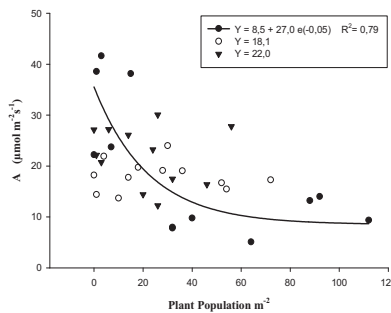


Figure 8. Photosynthesis rate (A - μmol m⁻² s⁻¹) of sugarcane varieties (●) RB72454, (○) RB857515 and (▼) SP80-1816 under competition with populations of *Brachiria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09.

A reduction in the photosynthesis rate (A) for RB72454 was observed, with the increase in the density of the competitor, being the same not observed for the other varieties (Figure 8). Photosynthesis rate is limited, in this case, by the reduced availability of CO₂ in the inner space of the leaf due to the lower influx of CO₂, as observed for Ci and Gs. However, some plants are more efficient in the stomatal adjustment, so that even with a small reduction in water availability there is not necessarily a reduction in the photosynthesis rate [9].

Some environmental factors impact the stomatal regulation, such as water stress, because highly negative water potentials induce stomatal closure with a consequent reduction in stomatal conductance [9], reducing the influx of CO₂ to the leaves and limiting the photosynthesis by deficiency of substrate. The nutritional deficiency may also lead to deficiency, for example, of proteins, enzymes and nutrients, such as potassium, which acts directly on the stomatal adjustment, and such deficiency could limit the photosynthesis rate [14]. From the results it is unlikely that the photosynthetic activity of plants was limited by the interference of radiation, or by the smaller change in the proportion of red and far-red light, because in this case, there would most likely be no changes in Ci and Gs [9].

The water use efficiency (WUE) is characterized as the amount of water lost by transpiration while a certain amount of CO₂ is captured for dry mass accumulation. Thus, plants, which present high photosynthesis rate while losing small amounts of water through transpiration, are considered more efficient in the use of water [9]. As the density of *B. brizantha* was increased, there was a reduction in WUE of the variety RB72454, with higher effect in the lower populations of the competitor (Figure 9). This may be an indicator that this variety, under competition with *Brachiaria*, is able to keep the photosynthesis rate under conditions of lower water availability. The other varieties presented a reduction in A compatible with the reductions observed in E, which resulted in stable WUE. Some researchers have observed that differences occur between the WUE in varieties of rice [43] and sugarcane [42] when subjected to both intra- and inter-specific competitions.

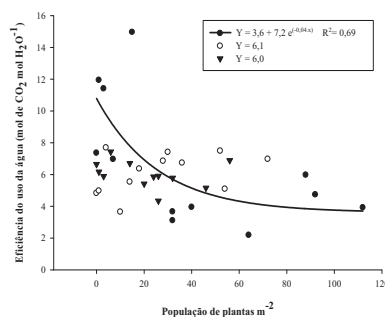


Figure 9. Water use efficiency (WUE - mol CO₂ mol H₂O⁻¹) of sugarcane varieties (●) RB72454, (○) RB857515 and (▼) SP80-1816 under competition with populations of *Brachiaria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09.

Based on the results, the infestation of *B. brizantha* interferes in the morphological components of sugarcane varieties, by reducing leaf area, shoot dry mass, diameter and number of stalks, plant height and number of leaves. The average yields of stalks for the three varieties studied showed a reduction of about 60 t ha⁻¹ when competing with the higher levels of infestation; the variety RB72454 was the most affected by competition with *B. brizantha*.

In relation to the physiological components, the variety RB72454 suffered the greatest effect by the competition for environmental resources, particularly for light and water, as the density of *B. brizantha* was increased. The photosynthesis rate and water use efficiency from sugarcane are limited as the competition with *B. brizantha* is increased, due to factors that lead to lower influx of CO₂ into the leaves of the crop, and the consequent higher transpiration. The varieties of sugarcane present differential susceptibility to the competition imposed by *B. brizantha*, and the variety RB72454 is highlighted as the most sensitive to competition among the tested.

8. Changes in physiological traits of sugarcane varieties under herbicide application

The experiment was installed in a randomized block design, with three replications in a factorial scheme 3 x 8. Factor A consisted of sugarcane varieties (RB867515, RB855156 and SP80-1816) and factor B by the herbicides tembotrione (Soberan® 200 mL ha⁻¹ + Aureo® 1,0 L ha⁻¹); MSMA (Volcane® 3,0 L ha⁻¹); diuron + hexazinone (Velpar-K GRDA® 2,0 kg ha⁻¹); sulfentrazone (Solara® 1,2 L ha⁻¹); trifloxysulfuron-sodium (Envoke® 30,0 g ha⁻¹+ Extravon® 0,2% v/v); tebuthiuron (Combine 500 SC® 2,0 L ha⁻¹); clomazone (Gamit® 3,0 L ha⁻¹), plus a check treatment with no herbicide.

The experimental units consisted of plastic pots containing 12 dm³ of substrate - Red Latosol, previously limed and fertilized according to the analysis: pH water 4.3; OM = 2.5 dag kg⁻¹; P = 1.5 mg dm⁻³; K = 40 mg dm⁻³; Al³⁺ = 0.5 cmol c dm⁻³; Ca²⁺ = 1.3 cmol c dm⁻³; Mg²⁺ = 0.2 cmol c dm⁻³; CTC(t) = 2.1 cmol c dm⁻³; CTC(T) = 6.39 cmol c dm⁻³; H+Al = 4.79 cmol c dm⁻³; SB = 1.6 cmol c dm⁻³; V = 25%; and clay = 38%. After filling out pot with the substrate, two buds of sugarcane were planted in each experimental unit. Ten days after emergence (DAE) only one plant was left per pot. Herbicide application was accomplished 50 DAE of sugarcane, at the stage of four to six fully expanded leaves. For the application, it was used a backpack sprayer, propelled by CO₂ and connected to a 2m wide bar with four nozzles Teejet TT 110.02, calibrated to spray 150 L ha⁻¹. Forty five days after application of the herbicides, physiological evaluations were done in the middle third of the first fully expanded leaf of the main tiller of the sugarcane plants. For that, an Infra Red Gas Analyzer (IRGA LCA Pro*) was used, in an open greenhouse, allowing free movement of the air. The photosynthesis rate (mol m⁻² s⁻¹), stomatal conductance of water vapor (Gs - mol m⁻¹ s⁻¹), transpiration rate (E - mol H₂O m⁻² s⁻¹) and the water use efficiency (WUE - mol CO₂ mol H₂O⁻¹) were obtained. The evaluations were conducted between 7:00 and 10:00 am. All data was analyzed by the F-test, and when significant, means were grouped by the test of Scott-knot at 5% probability.

There was interaction between herbicides and varieties for the photosynthesis rate, stomatal conductance, transpiration rate and the water use efficiency. The variety RB867515, when sprayed with sulfentrazone, tebuthiuron or clomazone, showed lower transpiration rate (E) compared with the check with no herbicide (Table 2). It is noteworthy that these herbicides act directly or not in the photosynthetic apparatus of plants that are applied. Sulfentrazone inhibits the enzyme protoporphyrinogen oxidase (PROTOX), acting indirectly on chlorophyll synthesis in sensitive plants. Tebuthiuron is an inhibitor of photosystem II acting on D1 protein, and clomazone inhibits the synthesis of carotenoids, whose function is the protection of chlorophyll against excess of light [8].

Herbicide	Sugarcane Variety		
	RB867515	RB855156	SP80-1816
Tembotrione	2.66 Aa	2.57 Aa	2.62 Aa
MSMA	2.76 Aa	2.56 Aa	2.06 Bb
Diuron+hexazinone	2.87 Aa	2.54 Aa	3.05 Aa
Sulfentrazone	2.08 Bb	2.17 Bb	2.81 Aa
Trifloxysulfuron-sodium	2.70 Aa	2.24 Bb	1.82 Bb
Tebuthiuron	2.24 Bb	2.03 Bb	2.83 Aa
Clomazone	2.35 Ab	2.07 Ab	2.77 Aa
Check	2.85 Aa	2.87 Aa	2.72 Aa
CV (%)		14.20	

Table 2. Transpiration rate ($E - \text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) of sugarcane varieties under competition with populations of *Brachiria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09.

Means followed by the same letter, uppercase in the rows and lowercase in the columns, are included in the same group by the test of Scott-Knot at 5% probability.

The application of sulfentrazone, trifloxysulfuron-sodium, clomazone and tebuthiuron on the variety RB855156 caused reduction in E. For variety SP80-1816, however, only trifloxysulfuron-sodium and MSMA had negative impact (Table 2). Trifloxysulfuron-sodium is an inhibitor of the enzyme acetolactate synthase (ALS), with indirect effect on photosynthesis because it acts on the production of the branched-chain amino acids valine, leucine and isoleucine, responsible for the production of proteins in plants.

With regard to the water use efficiency (WUE), no differences were observed inside variety or herbicide (Table 3). The water use efficiency is characterized as the amount of water transpired by a plant to produce a certain amount of dry mass [8]. Thus, more efficient crops in the water use can produce bigger amounts of dry mass per gram of water transpired. The most efficient use of water is directly related to stomatal opening, therefore, while the plant absorbs CO_2 for

Herbicide	Sugarcane Variety		
	RB867515	RB855156	SP80-1816
Tembotrione	8.06 Aa	9.50 Aa	6.40 Aa
MSMA	6.36 Aa	5.65 Aa	6.29 Aa
Diuron+hexazinone	7.37 Aa	8.43 Aa	6.95 Aa
Sulfentrazone	5.10 Aa	6.88 Aa	8.69 Aa
Trifloxysulfurom-sodium	7.53 Aa	7.04 Aa	7.49 Aa
Tebuthiuron	7.88 Aa	7.95 Aa	6.66 Aa
Clomazone	7.28 Aa	6.14 Aa	6.64 Aa
Check	10.12 Aa	8.41 Aa	9.56 Aa
CV (%)	22.62		

Table 3. Water use efficiency (WUE - mol CO₂ mol H₂O⁻¹) of sugarcane varieties under competition with populations of *Brachiria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09.

photosynthesis, water is lost with variable intensity, following a gradient of water potentials from the leaf surface to its surrounding air [44].

Means followed by the same letter, uppercase in the rows and lowercase in the columns, are included in the same group by the test of Skott-Knott at 5% probability.

With regard to the photosynthesis rate (A), it was observed that only sulfentrazone affected the variety RB867515, while the remaining treatments did not differ from the check (Table 4). Another research [45] evaluated varieties of sugarcane and found that RB867515 was the least affected by the application of commercially formulated mixture ametryn + trifloxysulfuron-sodium in greenhouse.

The influx of CO₂ in the leaf may be compromised due to the action of herbicides which inhibit the enzyme PROTOX, because there is formation of nitric oxide by the reactive oxygen species (ROS). This oxide stimulates the synthesis and activity of abscisic acid (ABA) - hormone that acts regulating stomatal closure [2]. Stomatal closure can also occur by the direct action of ROS, favoring the accumulation of calcium in the cytosol [46], or due to peroxidation of membranes of cells adjacent to stomata.

For the variety RB855156, MSMA, sulfentrazone, trifloxysulfuron-sodium, tebuthiuron and clomazone caused a reduction in A (Table 3). For SP80-1816, diuron + hexazinone and sulfentrazone did not affect this variable. It is noteworthy that hexazinone + diuron in mixture, even both being photosystem II inhibiting herbicides, did not cause reductions in A for the three varieties compared to control (Table 3). [42], working with six sugarcane varieties, found that under application of ametryn, RB855113 presented A similar to the control treatment, and the other varieties showed a decrease in this parameter under application of ametryn.

Means followed by the same letter, uppercase in the rows and lowercase in the columns, are included in the same group by the test of Scott-Knot at 5% probability.

Herbicide	Sugarcane Variety		
	RB867515	RB855156	SP80-1816
Tembotrione	22.10 Aa	24.48 Aa	16.45 Ab
MSMA	17.54 Aa	14.51 Ab	13.75 Ab
Diuron+hexazinone	21.15 Aa	21.17 Aa	21.22 Aa
Sulfentrazone	10.56 Ba	15.59 Bb	24.94 Aa
Trifloxysulfurom-sodium	20.53 Aa	15.85 Ab	13.90 Ab
Tebuthiuron	17.86 Aa	16.25 Ab	18.64 Ab
Clomazone	16.89 Aa	12.96 Ab	18.45 Ab
Check	28.44 Aa	24.07 Aa	26.03 Aa
CV (%)		27.78	

Table 4. Photosynthesis rate (A - $\mu\text{mol m}^{-2} \text{s}^{-1}$) of sugarcane varieties under competition with populations of *Brachiria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09.

When evaluating varieties under each herbicide, there was no correlation between them for all the products tested, except for sulfentrazone applied over SP80-1816, where there was higher A compared to the other varieties under the same herbicide (Table 4). [42] observed that the photosynthetic rate in sugarcane plants treated with trifloxysulfuron-sodium was similar to the respective control without herbicide application for all varieties evaluated in this study. Most of the herbicides used in this work, except MSMA and trifloxysulfuron-sodium, affect directly the photosynthesis rate because they have as a site of action either the chloroplast, inhibiting the transport of electrons, or the synthesis of chlorophyll, also interfering in the synthesis of pigments responsible for protecting the photosynthetic apparatus.

In RB867515 and SP80-1816, herbicides did not cause differences for stomatal conductance (Gs), but for RB855156, Gs decreased in the presence of all herbicides (Table 5). When evaluating the Gs of varieties within each herbicide, there was no difference among them. However, in the absence of herbicide, RB855156 showed greater Gs than the other two varieties (Table 5). Reduction of stomatal conductance was reported in soybean and *Portulaca oleracea* six hours after application of lactofen, a PROTOX inhibitor [47]. This product may promote stomatal closure due to oxidation processes and the concentration of nitric oxide, as already mentioned, which promotes formation of ABA, which in turn is a hormone that regulates the stomatal closure [2].

Means followed by the same letter, uppercase in the rows and lowercase in the columns, are included in the same group by the test of Scott-Knot at 5% probability.

According to the results, the herbicides tested affected differently the physiological traits of the three sugarcane varieties evaluated. Overall, the variety RB867515 showed the lowest variation in the photosynthesis rate in the presence of the herbicides, compared to the check.

Herbicide	Sugarcane Variety		
	RB867515	RB855156	SP80-1816
Tembotrione	0.67 Aa	0.46 Ab	0.48 Aa
MSMA	0.60 Aa	0.46 Ab	0.28 Aa
Diuron+hexazinone	0.74 Aa	0.44 Ab	0.70 Aa
Sulfentrazone	0.27 Aa	0.34 Ab	0.59 Aa
Trifloxysulfurom-sodium	0.52 Aa	0.31 Ab	0.20 Aa
Tebuthiuron	0.33 Aa	0.28 Ab	0.56 Aa
Clomazone	0.36 Aa	0.27 Ab	0.57 Aa
Check	0.61 Ba	1.00 Aa	0.47 Ba
CV (%)		46.9	

Table 5. Stomatal conductance ($G_s - \text{mol m}^{-1} \text{s}^{-1}$) of sugarcane varieties under competition with populations of *Brachiria brizantha* DFT/UFV, Viçosa-MG, Brazil, 2008/09.

The varieties SP80-1816 and RB855156 presented lower photosynthesis rates under application of most of the herbicides evaluated. It was also observed a decrease in stomatal conductance of the variety RB855156 after application of all herbicides.

9. Influence of herbicides on the photosynthetic activity of sugarcane genotypes

According to [48], the application of late post emergence herbicides in sugarcane fields may result in high toxicity to the crop, limiting yields. These authors attribute this to physiological changes in sugarcane plants, which would result in negative effects, also on the quality of the harvest. [42] studied the following herbicides, applied over several sugarcane varieties: ametryn - 2000 g ha^{-1} ; trifloxysulfuron-sodium - 22.5 g ha^{-1} ; and a commercial mixture containing ametryn + trifloxysulfuron-sodium at $1463 + 37.0 \text{ g ha}^{-1}$, respectively. Treatments were compared against a check with no herbicide. Results are summarized in Table 6.

In general terms, the CO_2 consumed by photosynthesis (ΔC) was smaller in treatments including the herbicide ametryn. There were also remarkable differences among varieties. The ΔC is directly related to the photosynthesis rate of the plant by the time of the evaluation. In this situation, it is possible to observe that the variety RB72454 and SP80-1816 were less susceptible to ametryn than the other genotypes.

The concentration of CO_2 within the leaf (C_i) was affected by the herbicide treatments, being also observed once more differences among genotypes. As expected, this parameter presented, in general terms, opposite behavior in comparison to ΔC . The application of the herbicide

Treatment	Sugarcane Genotype					
	RB72454	RB835486	RB855113	RB867515	RB947520	SP801816
Shoot Dry Mass (g plant⁻¹)						
TC	A 5.77 a	A 5.46 a	A 4.93 a	A 5.02 a	A 5.02 a	B 2.46 a
HA	B 2.83 b	B 3.69 ab	B 2.89 b	A 5.97 a	B 3.38 ab	B 3.63 a
HB	A 5.21 ab	B 2.81 b	B 3.81 ab	B 2.99 b	B 2.03 b	B 2.30 a
HC	A 2.90 b	A 1.99 b	A 2.46 ab	A 2.09 b	A 1.66 b	A 1.30 a
Consumed CO₂ – ΔC (μmol mol⁻¹)						
TC	A 124 ab	AB 149 a	B 120 a	AB 139 a	A 177 a	B 117 a
HA	A 109 b	B 75 c	AB 105 b	AB 91 b	AB 102 b	A 110 a
HB	AB 119 ab	B 106 b	AB 114 ab	AB 118 ab	A 144 ab	B 108 a
HC	A 131 a	A 107 b	AB 112 ab	A 95 b	A 118 b	A 111 a
Internal CO₂ Concentration – Ci (μmol mol⁻¹)						
TC	AB 102 a	A 177 a	AB 104 b	AB 123 b	B 68 c	AB 120 b
HA	A 165 a	A 136 ab	A 169 a	A 178 a	A 134 a	A 157 a
HB	AB 126 ab	AB 127 ab	AB 128 ab	AB 114 b	B 88 b	A 137 ab
HC	AB 146 ab	B 87,7 b	AB 125 ab	A 179 a	AB 145 a	A 158 a
Photosynthesis Rate – A (μmol m⁻² s⁻¹)						
TC	AB 45.1 a	AB 51.2 a	B 41.3 a	AB 47.9 a	A 60.7 a	AB 48.0 a
HA	A 37.5 b	B 25.8 b	A 36.1 a	B 28.9 b	A 37.0 b	A 37.3 b
HB	B 41.1 ab	B 36.6 ab	B 38.9 a	B 40.3 ab	A 49.5 ab	B 38.8 b
HC	A 42.1 ab	A 36.4 ab	A 38.4 a	B 32.8 b	A 40.5 b	A 40.1 b

Table 6. Physiological variables evaluated in sugarcane genotypes as a function of herbicide treatment. **TC:** control with no herbicide; **HA:** ametryn at 2000 g a.i. ha⁻¹; **HB:** trifloxysulfuron-sodium at 22.5 g a.i. ha⁻¹; **HC:** ametryn + trifloxysulfuron-sodium at 1463 + 37.0 g a.i. ha⁻¹. Means followed by the same letter at the column, inside each variable, are not different by the DMRT test at 5% probability.

ametryn, a photosynthesis II (PSII) inhibitor, resulted in higher concentrations of CO₂ within the leaf, once the photosynthesis of the genotypes under application of this herbicide was more severely affected. The CO₂ concentration within the leaf was about 50% higher in treatments involving ametryn than to the check with no herbicide. Trifloxysulfuron-sodium also caused changes in Ci, but not at the same magnitude of ametryn.

In general terms, the photosynthesis rate (A) observed at the treatment with trifloxysulfuron alone was similar to the control with no herbicide. On the same way, treatments involving the PSII inhibitor presented photosynthesis rate inferior to rates observed at the control treatment. When considering the treatment containing ametryn + trifloxysulfuron, it was possible to highlight the variety RB947520. The authors report that, even the damages caused by ametryn being more easily identified by parameters associated to the photosynthesis, variations due to the application of trifloxysulfuron-sodium were also detectable by changes in these parameters by using an Infra Red Gas Analyzer (IRGA). In other words, herbicide damage on crops can be effectively quantified by evaluating direct and indirect damage to the photosynthetic route. Furthermore, the accumulation of dry mass did not correlate directly with most of the studied physiological parameters, because plant growth is a result of biomass accumulation since the emergence until the moment of the evaluation. In this way, the authors remark the importance of evaluating both types of variables, physiological and biomass/growth-related, before concluding about the efficacy or impact of a given herbicide treatment. In addition, the authors remark the existence of differences among varieties in terms of susceptibility to herbicides, which were effectively identified by physiological parameters.

10. Conclusions

Both the abiotic and biotic factors, represented in this study respectively by the environmental conditions and the presence of a competitor, present impact on the sugarcane crop. This impact is differential on distinct varieties, and some should be preferred over others in situations where the types of stress differ. Sugarcane varieties to be planted should be chosen, besides commercial traits, based on their ability to avoid or to overcome the negative impact of the stress to which they are submitted. This will help reaching high yields under field conditions.

Brachiaria brizantha interferes both in the morphological and physiological components of sugarcane varieties. The variety RB72454 was the most affected by competition with *B. brizantha*, both in the morphological and physiological components. Thus, in areas with high infestation of weeds, there is an indication that the variety RB72454 should be avoided; if this variety is going to be used due to other positive traits, high levels of weed control are demanded in such fields.

Under application of herbicides, the variety RB867515 showed to be less sensitive to most of the herbicides tested, compared to SP80-1816 and RB855156. Herbicide damage to sugarcane can be effectively quantified by using an IRGA. Furthermore, the accumulation of dry mass usually did not correlate directly with most of the studied physiological parameters.

It is highlighted the importance of evaluating both types of variables, physiological and biomass/growth-related, before concluding about the efficacy or impact of a given herbicide treatment. It is also remarkable the existence of differences among sugarcane varieties in terms of susceptibility to herbicides, which were effectively identified by physiological parameters.

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