

Physiologic characteristics of corn and *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster in intercropping cultivation

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Competition between weeds and crops results in a lower availability of some resources to crop species, which causes deficiencies, such as water and nutritional deficiencies and low light quality or quantity. The aims of this study were to evaluate the effects of *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster, cv. Xaraés (brachiaria) emergence times comparing with corn (*Zea mays* L.), in addition to the fertilization and application of different nicosulfuron and atrazine herbicides levels, on the physiological characteristics of intercropped species. The physiological characteristics *Brachiaria* plants that were treated with herbicides were negatively affected. The emergence time of *Brachiaria* plants in comparison with corn altered the physiological characteristics of both. The *Brachiaria* plants that emerged before corn presented higher photosynthetic and transpiration rates and reduced the intensity of these characteristics in corn. Thus, the *Brachiaria* plants that emerged before the corn crop presented a higher physiological efficiency, and they were more competitive than the weeds that emerged during other seasons.

Key words: Atrazine, competition, fertilizing, intercropping, nicosulfuron, *Zea mays*.

INTRODUCTION

Plant competition is one of the primary biotic limiting factors in corn (*Zea mays* L.) crop yield, and it leads to high yield losses in some cases. Several weed species can infest corn crops, *Brachiaria* among them (Silva et al., 2007). *Brachiaria* plants belong to the corn family (*Poaceae*), and for that reason, they have similar demands for environment resources, which can interfere in the development of the agricultural crop (Radosevich et al., 1997).

Among the resources for which plants compete, water is considered to be one of the most limiting to production, primarily during hot and low rainfall seasons. Under these conditions, an important characteristic of cultivated plants can be affected, namely the water use efficiency

(WUE). The WUE is defined as the amount of water that is transpired by a plant to produce a certain amount of DM (Silva et al., 2007).

The competitive potential of plants for environmental resources varies according to the species present in an area (Rigoli et al., 2008), the population level (Vidal et al., 2004), the time of emergence of grass forage in relation to the crop (Silva et al., 2007), and the competitive characteristics of the cultivars (Galon et al., 2007). Competition between weed plants and crops leads to a lower supply of some resources to both species, causing deficiencies that culminate in alterations to the physiological characteristics associated with photosynthesis, such as water (Floss, 2008) and nutritional (Melo et al., 2006) deficiencies, and low light quality or quantity (Sharkey and Raschke, 1981). These limitations may lead to alterations in stomatal conductance, internal gas concentration and, consequently, in the photosynthetic activity and WUE. The most efficient water use is directly associated with the stomatal opening time; as carbon dioxide (CO₂) penetrates the leaf, water is lost by transpiration with variable intensity, depending on the potential gradient between the leaf surface and the atmosphere (Concenço et al., 2007; 2009).

Corn is considered to be a strong competitor with small plants, such as *Brachiaria*, primarily because of their size advantage over forage plants, as evidenced by the higher DM accumulation rate produced during their initial

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development stages (Silva et al., 2007). Corn presents an elevated interception capacity for photosynthetically active radiation along its canopy, reducing the quantity of this resource to other species. This interception varies according to the morphological characteristics of the cultivar, such as the plant height and leaf formation, in addition to the selected population (Freitas et al., 2008), crop development and interference from other species. However, studies reporting on physiological alterations in intercropped plants are scarce.

Thus, this study aimed to evaluate the effects of the *Brachiaria* emergence time in relation to that of corn, in addition to the effects of fertilization and nicosulfuron and atrazine herbicides on the physiological characteristics of corn and *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf, cv. Xaraés in intercropped cultivation.

MATERIALS AND METHODS

This study was conducted in a greenhouse at the Federal University of Viçosa (UFV, Viçosa, Minas Gerais) in a randomized complete block design, with four replicates in a (2 × 2 × 3) factorial scheme as follows: a) with and without herbicides; b) total fertilization and 100% recommended fertilization for pot cultivation; and c) three emergence seasons of *Urochloa brizantha* 'Xaraés' (*Brachiaria*) in comparison with corn. During season 1, *Brachiaria* emerged 10 d before corn; during season 2, *Brachiaria* and corn emerged at the same time; and during season 3, *Brachiaria* emerged 10 d after corn.

The experimental units were constituted of punched bottom pots containing 20 dm³ substrate (soil + fertilizers). A red-yellow Latosol was used as the substrate, and it had been previously corrected and fertilized with 20 g sodium chloride, 500 g simple superphosphate, 50 g ammonium sulfate, and 150 g dolomitic limestone, as recommended by Cantarutti et al. (2007) for pot fertilization.

According to the analysis, the soil presented the following characteristics: pH in water 4.3, organic matter: 2.5%, 1.5 mg P dm⁻³, 40 mg K dm⁻³, 0.5 cmol_c Al³⁺ dm⁻³, 1.3 cmol_c Ca²⁺ dm⁻³, 0.2 cmol_c Mg²⁺ dm⁻³, cation exchange capacity effective (CTC) = 2.1 cmol_c dm⁻³, CTC = 6.39 cmol_c dm⁻³, H+Al = 4.79 cmol_c dm⁻³, base saturation = 1.6 cmol_c dm⁻³, Al saturation = 25%, and 38% clay.

After filling pots with substrate, two corn seeds and six *Brachiaria* seeds were sowed per pot, and resulting seedlings were subsequently thinned to one corn and three *Brachiaria* plants. The pots were maintained in a protected environment inside a greenhouse. Irrigation was performed daily to maintain the soil at approximately 80% field capacity. Preliminary germination tests were performed for different forage emergence times in relation to corn. The tests uncovered an interval of 10 d between sowing and *Brachiaria* emergence and 5 d for corn emergence. To following different *Brachiaria* germination times in relation to corn were identified: 1) *Brachiaria* emergence

10 d before corn = corn sowing 5 d after *Brachiaria* emergence; 2) *Brachiaria* emergence at the same time as corn = corn sowing 5 d after *Brachiaria* sowing; and 3) *Brachiaria* emergence 10 d after corn = *Brachiaria* sowing on the day of corn emergence. The application of the herbicides atrazine (Atrazinex, 6-chloro-N₂-ethyl-N₄-isopropyl-1,3,5-triazine-2,4-diamine at 4 L ha⁻¹) and nicosulfuron (Sanson, 2-[(4,6-dimethoxypyrimidin-2-yl carbamoyl) sulfamoyl]-N,N-dimethylnicotinamide 150 mL ha⁻¹) was performed 21 d after corn emergence when crop plants presented four completely expanded leaves. For the herbicide applications, a coastal pulverizer pressurized with CO₂ at 3.0 kgf cm⁻² was equipped with a bar and a pulverization tip (TT 110.02 series) and calibrated to apply 200 L ha⁻¹ herbicide syrup.

The physiological evaluations were performed during the middle third of the first completely expanded leaf of the *Brachiaria* primary tillage and in the first completely expanded corn leaf at 15 and 45 d after herbicide application. An infrared gas analyzer was used (IRGA LCA Pro+, ADC BioScientific, Hoddesdon, UK) for these evaluations in an open greenhouse, allowing for free air circulation. The stomatal conductance to water vapor (Gs, mol m⁻² s⁻¹), transpiration (E, mol H₂O m⁻² s⁻¹) and photosynthetic (A, μmol m⁻² s⁻¹) rates were evaluated. In addition, the water use efficiency (WUE, mol CO₂ mol⁻¹ H₂O) was obtained by studying the relation between the CO₂ concentration that was fixed by photosynthesis and the transpired water during the same period, in addition to the internal C/atmospheric C ratio (Ic/Ac). Evaluations were performed between 07:00 and 10:00 h.

Data were subjected to ANOVA and the means were compared by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

At 15 d after herbicide application, the *Brachiaria* plants that were treated with a nicosulfuron and atrazine mixture presented a lower transpiration rate (E) when compared with treatments without the mixture (Table 1). Nicosulfuron is an inhibitor of the acetolactate synthase enzyme (ALS), which is responsible for the synthesis of branched amino acids (leucine, isoleucine, and valine), and atrazine is responsible for inhibiting photosystem II, which paralyzes the flow of electrons in the chloroplast

Table 1. Photosynthetic activity (A, mol m⁻² s⁻¹), transpiration (E, mol H₂O m⁻² s⁻¹), stomatal conductance (Gs, mol m⁻² s⁻¹), water use efficiency (WUE, mol CO₂ mol⁻¹ H₂O) and the internal and external CO₂ concentration ratio (Ic/Ac) in *Brachiaria* plants as evaluated 15 d after atrazine + nicosulfuron herbicide applications.

Herbicide	A	E	Gs	WUE	Ic/Ac
With herbicides	8.20b	3.02b	0.16b	2.70b	0.57a
Without herbicides	23.10a	4.36a	0.25a	5.30a	0.54b
CV, %	26.32	22.33	41.55	23.40	11.04

Means followed by same letter in the column do not differ according to the F test at 5% probability.

transport chain. Thus, it is believed that these herbicides interfere in E, given that atrazine acts directly and nicosulfuron interferes indirectly in the photosynthetic apparatus.

Reductions in the transpiration rate are associated with stomatal closure, and variations in stomatal opening cause alterations in the water potential (Brodribb and Hill, 2000). A plant tends to close its stomata when light levels are below photosynthetically active radiation, or to avoid water stress (Cochard et al., 2002). All these parameters are connected by a cost/benefit relation because E is also a leaf temperature reduction mechanism. Transpiration and CO₂ uptake processes only occur when the stomata are open, in addition to stomatal conductance (Gs). Because of the latent heat of evaporation (heat is effectively used to “heat” water and allow evaporation), transpiration has a powerful cooling effect, which is important for leaf temperature regulation (Farquhar and Raschke, 1978).

The presence of herbicides reduced the Gs of *Brachiaria* plants (Table 1). This finding is also possibly explained by stomatal closure, which is influenced by several factors, such as the water availability, light and energy, and pollution and herbicides used for weed control.

A reduction in the stomatal conductance was observed in soybean (*Glycine max* [L.] Merr.) and portulaca-weed (*Portulaca oleracea* L.) 6 h after applying lactofen, which is a protox enzyme inhibitor (Wichert and Talbert, 1993). Foliar conductance is partially composed of epidermis cuticle conductance and, when stomata are open, by Gs as controlled by guard cells in the stomata. Thus, Gs is proportional to the stomata number, size, and opening diameter; these characteristics depend on other endogenous and environmental factors in addition to the previously mentioned factors (Brodribb and Holbrook, 2003). *Brachiaria* plantlets are mostly considered to be sensitive to nicosulfuron applications that are performed during early post-emergence in recommended commercial doses, and the symptoms of sensitive plants are foliar chlorosis, necrosis, and growth reduction (Lorenzi, 2000; Shim et al., 2003).

Brachiaria plants submitted to herbicide mixtures presented a lower photosynthetic rate (A) than those that did not receive herbicide treatment (Table 1). This reduction can be explained by the interference of herbicides in the plant photosynthetic apparatus. The higher Ic/Ac ratio in herbicide-treated plants indicates that plants fixed low CO₂ concentrations in the substomatal chamber, showing that photosynthesis reduction is a result of damages during the photosynthetic process (Table 1). Ogliari et al. (2009) noticed that the mesotrione herbicide reduced the photosynthetic rate in corn plants by 78% on the first, 66% on the second, and 75% on the third day after application relative to the control.

When comparing times of emergence, *Brachiaria* plants that emerged 10 d before corn exhibited a higher

E than the *Brachiaria* from treatments with simultaneous and subsequent emergence relative to corn (Table 2). Plants that emerged 10 d after corn presented three leaves during this phase, were younger and, consequently, more sensitive to herbicide action, in addition to engaging in less competition with the crop. However, if *Brachiaria* is not correctly managed, corn production will be compromised and it will not be possible to remove the weeds without causing severe damage to the agricultural crop. The alternative is to apply sub-doses of nicosulfuron to delay *Brachiaria* growth without damaging the corn too much. The dosage depends on the *Brachiaria* growth stage and on weeds that occur in the area. The more developed the *Brachiaria*, the higher the recommended dosage (Ferreira et al., 2007).

In comparing treatments with and without herbicides within each *Brachiaria* emergence time, it was clear that the treatments with herbicide applications presented a lower A at all emergence times at 45 d after application (Table 3), demonstrating that herbicide applications promote a reduction in the A of *Brachiaria*, regardless of the weed’s developmental stage. However, herbicide applications promoted a higher photosynthesis reduction when *Brachiaria* plants emerged at the same time or after corn plants (Table 3), confirming the higher efficiency of herbicide application in younger plants.

Regarding the fertilizer levels within specific time periods, the photosynthesis was inferior in the treatment receiving half the fertilizer at 10 d after emergence

Table 2. Photosynthetic activity (A, mol m⁻² s⁻¹), transpiration (E, mol H₂O m⁻² s⁻¹), stomatal conductance (Gs, mol m⁻² s⁻¹), water use efficiency (WUE, mol CO₂ mol⁻¹ H₂O) and the internal and external CO₂ concentration ratio (Ic/Ac) of *Brachiaria* plants according to the emergence time on different days at 45 d after applying atrazine + nicosulfuron herbicides.

<i>Brachiaria</i> emergence	A	E	Gs	WUE	Ic/Ac
10 DBE	21.62a	4.12a	0.24ns	5.20a	0.57a
Concomitant	13.20b	3.25b	0.25	2.96b	0.59a
10 DAE	15.92b	3.69ab	0.24	3.57b	0.50b
CV, %	26.33	22.12	41.98	23.76	11.08

Means followed by same letter in the column do not differ according to Tukey’s test at 5% probability.

DBE: Days before corn emergence, DAE: days after corn emergence, ns: non-significant.

Table 3. The photosynthetic activity (A) of *Brachiaria* plants according to their emergence times relative to corn emergence, herbicide application and fertilization levels at 45 d after herbicide application.

<i>Brachiaria</i> emergence	Total fertilizer	Half fertilizer	With herbicide	Without herbicide
	mol m ⁻² s ⁻¹			
10 DBE	24.5aA	18.71b	16.52bA	26.83aA
Concomitant	12.9aB	13.62a	5.52bB	20.94aA
10 DAE	12.4bB	19.54a	3.99bB	27.91aA
CV, %			26.13	

Means followed by the same lower-case letter in the lines and capital letters in the columns do not differ according to Tukey’s test at 5% probability.

DBE: Days before corn emergence, DAE: days after corn emergence.

(DAE). This reduction may have been caused by a nutritional limitation. For *Brachiaria* plants that received the full dose of fertilizer, a lower A was observed when plants emerged 10 d after corn (Table 3). This reduction may have been caused by higher initial corn development, which was caused by previous emergence and greater nutrient availability.

The competition between weeds and crops is a critical factor for crop development when weed species sets at the same time or before the crop (Radosevich et al., 1997). However, if the crop is established first, it will be able to cover the soil quickly because of its cultivated species, energy, initial growth rate and seedling density, which will exclude or significantly inhibit the growth of weeds. Herbicide application in addition to *Brachiaria* emergence at the same time or after corn provide a photosynthetic rate reduction in forage, interfering in the competitive capacity of *Brachiaria* (Table 3).

The difference among fertilization levels within treatments with and without herbicides and for times within herbicide treatments was verified between levels within treatments. In Table 3, the competition factor is included because *Brachiaria* plants that emerged before corn presented a higher competitive capacity, which must be taken into consideration. However, the E was not affected under any fertilization level among treatments with and without herbicides. No differences were observed in the transpiration and WUE of *Brachiaria* plants intercropped with corn, for fertilization levels within treatments with and without herbicides and for emergence times within herbicide treatments (Table 4).

Regarding treatments with and without herbicides within each time period, there was no difference in the E and WUE between treated or untreated plants and those that emerged before corn. However, for plants that emerged simultaneously and after corn, the samples treated with herbicides presented lower E and WUE values (Table 4). For times within treatments that were with and without herbicide, plants that emerged 10 d before corn in the presence of herbicide exhibited lower E and WUE values in comparison with those that emerged simultaneously and after corn. No difference was noted

Table 4. The transpiration and water use efficiency of *Brachiaria* plants intercropped with corn according to emergence times when comparing corn emergence and herbicide application at 55 d after herbicide application.

<i>Brachiaria</i> emergence time	Transpiration		Water use efficiency	
	With herbicide	Without herbicide	With herbicide	Without herbicide
	mol H ₂ O m ⁻² s ⁻¹		mol CO ₂ mol ⁻¹ H ₂ O	
10 DBE	3.74aA	4.50aA	4.97aA	5.44aA
Concomitant	2.07bA	4.44aA	1.85bA	4.08aA
10 DAE	3.24bA	4.14aA	1.60bA	5.56aA
CV, %	20.5		23.0	

Means followed by same lower-case letter in line and capital letter in column do not differ according to Tukey's test at 5% probability.
DBE: Days before corn emergence, DAE: days after corn emergence.

between the emergence times of plants that did not receive herbicides applications (Table 4). When comparing the E and WUE in the presence and absence of herbicides between the different times of emergence, the treatments that received products presented lower E and WUE values when *Brachiaria* germinated simultaneously with corn or 10 d afterwards, indicating higher herbicide efficiency when applied to younger plants (Table 4).

Unlike *Brachiaria* plants that were treated with atrazine and nicosulfuron herbicides in sub-doses, the corn plants presented higher photosynthesis, E, stomatal conductance, and WUE values (Table 5). The improved physiological efficiency of corn plants is explained by the *Brachiaria* growth rate reduction caused by herbicide application, because the lower growth of *Brachiaria* plants reduces competition with corn plants for nutrients, primarily for water. The low photosynthetic rate presented by corn plants in the treatments without herbicide applications (Table 5) was most likely caused by a water deficit produced by competition with *Brachiaria* plants. The most significant plant response to water deficits consists of stomatal closure (Taiz and Zeiger, 2009). Plants frequently show physiological responses when exposed to water deficit situations, which results directly in water savings. Thus, stomatal conductance is directly associated with water availability in the soil (Bianchi et al., 2007). When evaluating the Ic/Ac ratio, the lower value was verified in treatments without herbicide application (Table 5). This finding indicates that the photosynthetic apparatus is working normally and that the E reduction is associated with a reduced CO₂ concentration in the substomatal chamber, which is caused by stomatal closure. The plants in treatments with herbicide applications presented higher stomatal conductance, which allows for higher CO₂ synthesis and increases the photosynthetic rate. This finding is evidenced by the higher Ic/Ac ratio, indicating that there is an appropriate CO₂ supply for the cells.

With regards to the *Brachiaria* emergence time, the corn plants presented lower E, A, stomatal conductance, and WUE in earlier *Brachiaria* emergence treatments (Table 6). The photosynthesis reduction in corn plants caused by early *Brachiaria* emergence is also associated with the competition process between plants and not to the direct interference of herbicides in the photosynthesis phases. *Brachiaria* plants that emerged before corn

Table 5. Photosynthetic activity (A, mol m⁻² s⁻¹), transpiration (E, mol H₂O m⁻² s⁻¹), stomatal conductance (Gs, mol m⁻² s⁻¹), water use efficiency (WUE, mol CO₂ mol⁻¹ H₂O) and the internal and external CO₂ concentration ratio (Ic/Ac) in corn plants as evaluated 15 d after atrazine + nicosulfuron herbicide application.

Herbicide	A	E	Gs	WUE	Ic/Ac
With herbicide	52a	1.40a	7.3a	7.0a	0.426a
Without herbicide	41b	0.82b	6.7b	5.9b	0.330b
CV, %	22.03	14.58	28.23	16.90	13.02

Means followed by same letter in column do not differ according to F test at 5% probability.

Table 6. Photosynthetic activity (A, mol m⁻² s⁻¹), transpiration (E, mol H₂O m⁻² s⁻¹), stomatal conductance (Gs, mol m⁻² s⁻¹), water use efficiency (WUE, mol CO₂ mol⁻¹ H₂O) and the internal and external CO₂ concentration ratio (Ic/Ac) in corn plants according to *Brachiaria* emergence times as evaluated 45 d after atrazine + nicosulfuron herbicide application.

<i>Brachiaria</i> emergence	A	E	Gs	WUE	Ic/Ac
10 DBE	36.12b	6.03b	0.51b	5.97b	0.290b
Concomitant	54.81a	7.41a	1.36a	7.03a	0.437a
10 DAE	52.97a	7.69a	1.50a	6.97a	0.419a
CV, %	22.03	14.58	28.23	16.90	13.02

Means followed by same letter in column do not differ according to Tukey's test at 5% probability.

DBE: Days before corn emergence, DAE: days after corn emergence.

obtained a competitive advantage over the agricultural crop, given that they were ready to absorb higher amounts of water and nutrients. Under moderate water deficiency, corn presents decreased photosynthesis because of a reduction in stomatal conductance, leading to stomatal closure and to transpiration reduction. Nevertheless, non-stomatal limitations (biochemistry) began to occur under severe and prolonged stress (Grzesiak et al., 2006). Although the water deficit has not been measured, there were visible symptoms of water deficit in corn plants that were cultivated in pots in which the forage emerged first, such as wilting and curling of the leaves. Under these conditions, the plants tended to close their stomata to minimize water loss to the environment. Stomatal closure reduces CO₂ entry into the substomatal chamber, reducing the quantity of available substrate for the Calvin cycle. This hypothesis can be confirmed by evaluating the Ic/Ac ratio, which makes a direct association between the CO₂ concentration in the substomatal chamber and the CO₂ concentration in the environment, so the lower Ic/Ac value in the treatment presenting *Brachiaria* emergence 10 d before corn indicates that corn plants are consuming CO₂ normally and that photosynthesis reduction is occurring because of the substrate reduction for photosynthesis.

These results demonstrate the importance of adequate crop seeding and fertilization in corn intercropped with *Brachiaria*, with the aim of achieving quick germination and development in the agricultural crop plants to the detriment of *Brachiaria* plants. In addition, the benefit of using sub-doses of atrazine and nicosulfuron herbicides in integrated cultivation systems is confirmed, because this regimen reduces the growth of *Brachiaria* plants and potentializes corn production.

CONCLUSIONS

Sub-doses of atrazine and nicosulfuron herbicides increased photosynthesis, stomatal conductance, transpiration, and water use efficiency rates in corn plants and reduced the same variables in *Brachiaria* plants. *Brachiaria* plants that emerged before corn presented higher photosynthesis, stomatal conductance,

transpiration, and water use efficiency values and reduced these parameters in corn plants.

The higher nutrient availability increased the photosynthetic rate of the crop that first developed in the intercropped system.

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