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## Adverse Effect of Bermudagrass on Physiological and Growth Components of Cotton

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**Abstract:** A two-year (2002/03 and 2003/04) experiment was conducted in the research site of the Agricultural University of Athens, Greece, to study the effects of Bermudagrass (*Cynodon dactylon* L.) in several agronomic and physiological characteristics of cotton (*Gossypium hirsutum* L.). Effects on cotton depended on timing of weed influence. Bermudagrass reduced stomatal aperture, chlorophyll content and chlorophyll fluorescence of cotton plants. Growth of cotton was also significantly reduced. Maximum reduction was 41.7% for stem height, 62.0% for root dry matter, 61.9% for stem dry matter and 34.7% for stem diameter. Stomatal resistance, chlorophyll content and chlorophyll fluorescence are proposed as suitable indicators that in addition to agronomic characteristics can reliably be used to measure adverse effects caused to cotton due to Bermudagrass interference.

**Key words:** Fv/Fm, allelopathy, stress, malnutrition, growth inhibition

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### INTRODUCTION

Interference between plants is a research field that receives growing interest. Both resource competition and allelopathy can be considered as parts of interference and essentially impossible to be distinguished in natural systems (Inderjit and Del Moral, 1997). Traditionally, agronomic characteristics are being measured to evaluate beneficial or (most commonly) harmful effects on plants when interfere with other plant species. Secondary metabolites (phenolics, organic acids) as well as plant extracts and/or exudates are tested for their effect on water relations and photosynthesis (Yu *et al.*, 2003) of target plants. Stomatal resistance and chlorophyll fluorescence are being measured to evaluate plant stress caused by abiotic factors (Hopkins, 1999; Lambers *et al.*, 1998; Hall *et al.*, 1995).

Weeds are known to be a major problem in crop husbandry. Early experimentation revealed that crops suffer enormously from weeds, which compete for light, air and nutritive substances, exhaust the moisture, harbor various insects and parasitic fungi, make cultural operations difficult and interfere with good germination and plant development. In the case of cotton, the plants are very delicate and if left unassisted may soon be overcome by weeds, either perishing or giving a negligible crop (Christidis and Harrison, 1955). It is reasonable to expect that losses are heavier when time of interference

exists in the first two months of growth, whereas after this period cotton becomes less vulnerable to weed infestation.

A major weed species in cotton fields in Greece is Bermudagrass (*Cynodon dactylon* L.). Preliminary experimentation in the research site of the Agricultural University of Athens, Greece, revealed significant influence of Bermudagrass on cotton growth (Bouchagier and Efthymiadis, 2003). In this study, the effect of Bermudagrass on vegetative growth and on important photosynthetic characteristics (stomatal resistance, chlorophyll concentration and Fv/Fm values) of cotton is studied.

### MATERIALS AND METHODS

The experiment was conducted in the experimental field (outdoors) of the Agricultural University of Athens (37°59'02" N; 23°42'08" E; 34 m alt.), Greece, from May to October in two consecutive years (2002, 2003). The site is characterized by dry summers, with high temperatures. Annual temperature and precipitation are 17.9°C and 434 mm, respectively.

Cotton seeds (cultivars Campo and Millenium) were sown in cylindrical pots (height-diameter 17 cm), in clay-loam soil (29.8% clay, 34.3% silt, 35.9% sand) taken from the topsoil of the experimental field. Cotton was sown by hand on 17 May (in 2002) and 20 May (in 2003).

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After plant emergence, seedlings were thinned to one seedling/pot. Bermudagrass rhizome fragments taken from stands of the experimental field were planted near the pot circumference (4 rhizome fragments/pot). Fragments were 4-6 cm long and contained 4-5 nodes each. Pots were randomly allocated to treatments. For each cultivar, rhizomes were planted at 0, 21, 42 Days After Sowing (DAS) and there were 12 pots with no rhizomes (control). During growth, plants were fertilized twice with Complisal Fluid 12-4-6 (N-P-K) enriched with micronutrients. Plants were watered in 1-2 days intervals. Consideration was taken to avoid water deficiency symptoms in cotton leaves. Pots were set in rows (8 rows, 12 pots/row) at 0.5 m apart on the row and 1 m apart between rows.

The experiment was held in pots to avoid risk due to possible variability in soil conditions if plants would grow in the field.

The effect of Bermudagrass on cotton growth was measured in both years of experimentation (2002/03 and 2003/04). Cotton plants were harvested on 25 October (in 2002) and 21 October (in 2003) when 80% of cotton bolls were open (stage 88 of BBCH scale) (BBCH, 2001). Then stem height and stem diameter (at the soil line) were measured. Further cotton roots were washed over a 5 µm mesh sieve to separate from soil and roots as well as stems were then oven dried at 70°C to a constant weight to measure stem and root Dry Matter (DM). Soil samples were taken from pots and N, P and K concentration, as well as pH, Electric Conductivity (EC) and organic matter of soil were measured according to Page *et al.* (1982).

Stomatal resistance of cotton plants was measured during the first year of the experiment. Measurements were taken from 13 August to 2 September 2002. In this period stomatal resistance was measured 14 times in regular (two days) intervals, between 11.00-13.00 h from the abaxial surface of intact and fully expanded leaves using a porometer (AP A4 Delta-T Devices-Cambridge-UK). Specifically on the days of measurements, plants were extra watered (two hours before the measurement), in order to completely cover water requirements.

A chlorophyll meter (Minolta SPAD-502, Minolta Co. Ltd., Japan) was used to take chlorophyll readings. Readings were taken three times on 22 August, 10 September and 2 October 2003 on fully expanded and intact functional leaves. In order to relate chlorophyll meter readings with chlorophyll content, a reference curve was created according to Lichtenthaler and Wellburn (1983). Chlorophyll content was then calculated according to Harborne (1988).

Chlorophyll fluorescence was measured by means of Fv/Fm (Fv: variable fluorescence; Fm: maximum

fluorescence) on intact and fully expanded leaves with a portable Fim 1500 fluorometer (ADC Ltd., UK). Measurements were taken between 13.00-15.00 h from the adaxial surface of cotton leaves. Before fluorescence measurements leaves were dark adapted for 30 min. Excitation of chlorophyll was achieved by supplying light of 1500 µmol m<sup>-2</sup> sec<sup>-1</sup> for 5 sec. Totally 6 measurements of fluorescence were made between 8 August to 9 September 2003 at regular intervals.

The experimental design was a Completely Randomised Design. For agronomic characteristics the experiment was analysed as factorial with three factors, A) year of planting (2 levels; 2002/03 and 2003/04), B) cotton cultivar (2 levels; cultivars Campo and Millenium) and C) time of rhizome planting (4 levels; 0, 21, 42 DAS and control). Total size of the experiment was 96 pots (2 cultivars × 4 treatments × 12 plants/cultivar/treatment). For stomatal resistance, chlorophyll concentration and chlorophyll fluorescence the experiment was analysed as factorial with two factors, A) cotton cultivar (cultivars Campo and Millenium), B) time of rhizome planting (0, 21, 42 DAS and control). Prior to statistical analysis, data were tested for homogeneity of variances and for normal distribution by the Shapiro-Wilk test. Stem height, stem dry matter, stomatal resistance, chlorophyll concentration and Fv/Fm values were given a Box-Cox transformation before conducting the ANOVA test. For agronomic characteristics significant differences between treatments were identified using Student t-test (Federer, 1955) with one degree of freedom (df) for year of planting, one df for cotton cultivar and three df for time of rhizome planting. For stomatal resistance, chlorophyll content and Fv/Fm values, the Repeated Measurements Design was applied and means were compared with F-test with one df for cotton cultivar and three df for time of rhizome planting. All comparisons were made at 5% level of significance. Analysis was performed with the statistical software JMP 5.1 (2003).

## RESULTS AND DISCUSSION

Yield losses of crops depend heavily on time of weed emergence (Kropff *et al.*, 1992). When weeds and crop emergence is synchronized, a crop suffers higher losses than if a crop has a clear time precedence (Swanton *et al.*, 1999). Thus it is useful to know the length of time where crops must grow free of weeds, so as not to be suppressed by competition (Van Acker *et al.*, 1993; Knezevic *et al.*, 1994). Cotton is a crop exceedingly sensitive to the competition of weeds (Christidis and Harrison, 1955). Presence of weeds for more than 3 weeks

Table 1: p-values of pair wise comparison of treatments for stomatal resistance, chlorophyll content and Fv/Fm values of cotton plants when influenced by Bermudagrass at 0, 21, 42 DAS

Parameters	Control-0 DAS	Control-21 DAS	Control-42 DAS	0-21 DAS	0-42 DAS	21-42 DAS
Stomatal resistance	<0.0001	0.0088	0.0427	0.0592	0.0118	0.5350
Chlorophyll content	<0.0001	0.0002	0.0805	0.3911	0.0030	0.0346
Fv/Fm	0.0029	0.0623	0.5217	0.1781	0.0003	0.0102

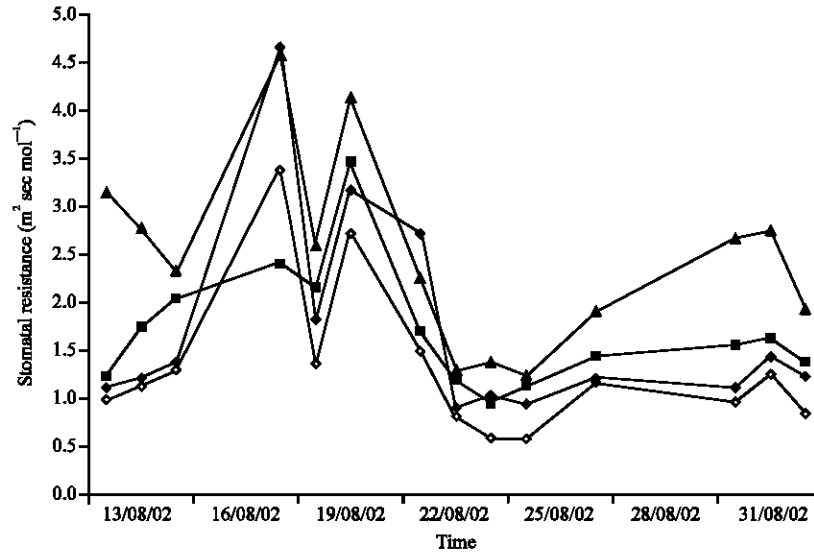


Fig. 1: Stomatal resistance of cotton cultivar Campo when influenced by Bermudagrass at 0 (▲), 21 (■), 42 (◆) DAS and control (○)

after cotton emergence can cause significant reduction in crop growth and lint yields (Papamichail *et al.*, 2002). *Cynodon dactylon* is an injurious weed species worldwide. It is quoted as one of the world's worst weeds although in some places it is better known as a valuable lawn grass or as a hay and fodder crop. Bermudagrass is frequently found in Europe in vineyards and orchards and in cotton fields in East Mediterranean region (Boz, 2000).

In this study, Bermudagrass caused increase in stomatal resistance of cotton plants ( $p < 0.0001$ ) whereas cultivars were found to be equally susceptible ( $p = 0.5244$ ) to weed effect. Interaction cultivars  $\times$  treatments was not significant ( $p = 0.9835$ ). Pair wise comparison revealed significant differences between treatments (Table 1).

Increase in stomatal resistance was scaling and depended on time of rhizome planting. Inferior stomatal resistance was measured in control, followed in ascending order by 42, 21 and 0 DAS. For cultivar Campo during the course of measurements, stomatal resistance ranged between 0.58-3.4 ( $m^2 \text{ sec mol}^{-1}$ ) in control, 1.24-4.55 ( $m^2 \text{ sec mol}^{-1}$ ) in 0 DAS, 0.95-3.46 ( $m^2 \text{ sec mol}^{-1}$ ) in 21 DAS and 0.9-4.65 ( $m^2 \text{ sec mol}^{-1}$ ) in 42 DAS treatment (Fig. 1). For cultivar Millenium, stomatal resistance at the same period ranged between 0.6-2.21 ( $m^2 \text{ sec mol}^{-1}$ ) in control, 1.14-4.3 ( $m^2 \text{ sec mol}^{-1}$ ) in 0 DAS, 0.8-2.93 ( $m^2 \text{ sec mol}^{-1}$ ) in 21 DAS and 0.82-4.71 ( $m^2 \text{ sec mol}^{-1}$ ) in 42 DAS treatment (Fig. 2).

Chlorophyll meter readings were related with total chlorophyll content with a reference ( $y = 0.2811x - 1.973$ ;  $R^2 = 0.5963$ ). Bermudagrass caused reduction in total chlorophyll content of cotton ( $p < 0.0001$ ). Significant differences were found between cultivars ( $p = 0.0145$ ). Interaction cultivars  $\times$  treatments was not significant ( $p = 0.3547$ ). Pair wise comparison revealed significant differences between treatments (Table 1).

Decrease in chlorophyll content of cotton was scaling and depended on time of rhizome planting. Inferior values of chlorophyll content were measured when rhizomes were planted at 0 DAS, followed in ascending order in treatments 21 DAS, 42 DAS and control. In Campo plants, chlorophyll content ranged between 6.75-8.04  $mg \text{ cm}^{-2}$  in 0 DAS, 7.01-8.44  $mg \text{ cm}^{-2}$  in 21 DAS, 7.62-9.67  $mg \text{ cm}^{-2}$  in 42 DAS and 8.15-10.15  $mg \text{ cm}^{-2}$  in control (Fig. 3). In Millenium plants, chlorophyll content ranged between 7.21-7.39  $mg \text{ cm}^{-2}$  in 0 DAS, 7.20-7.39  $mg \text{ cm}^{-2}$  in 21 DAS, 7.19-8.34  $mg \text{ cm}^{-2}$  in 42 DAS and 8.06-8.7  $mg \text{ cm}^{-2}$  in control (Fig. 4).

Decrease of Fv/Fm values of cotton was observed when plants were influenced by Bermudagrass ( $p = 0.0011$ ). Significant differences were also found between cultivars ( $p = 0.0029$ ). Interaction cultivars  $\times$  treatments was not significant ( $p = 0.1837$ ). Pair wise comparison revealed significant differences between treatments (Table 1).

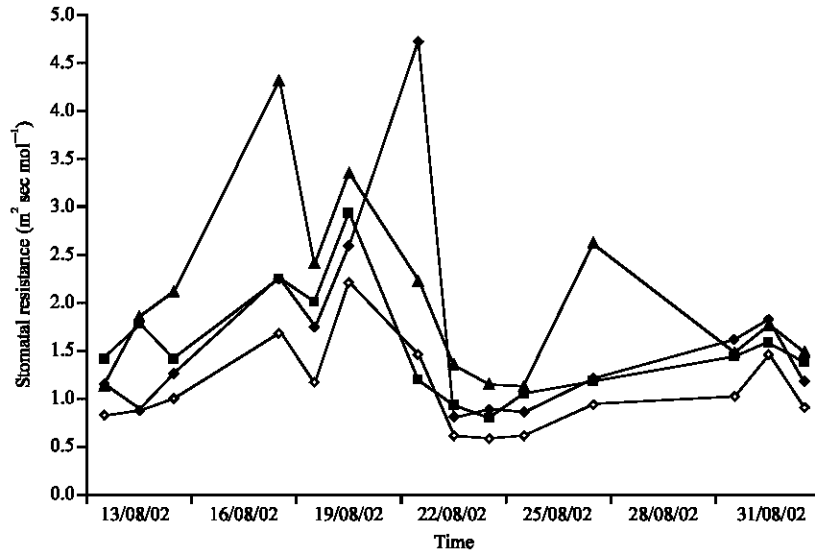


Fig. 2: Stomatal resistance of cotton cultivar Millenium when influenced by Bermudagrass at 0 (▲), 21 (■), 42 (◆) DAS and control (○)

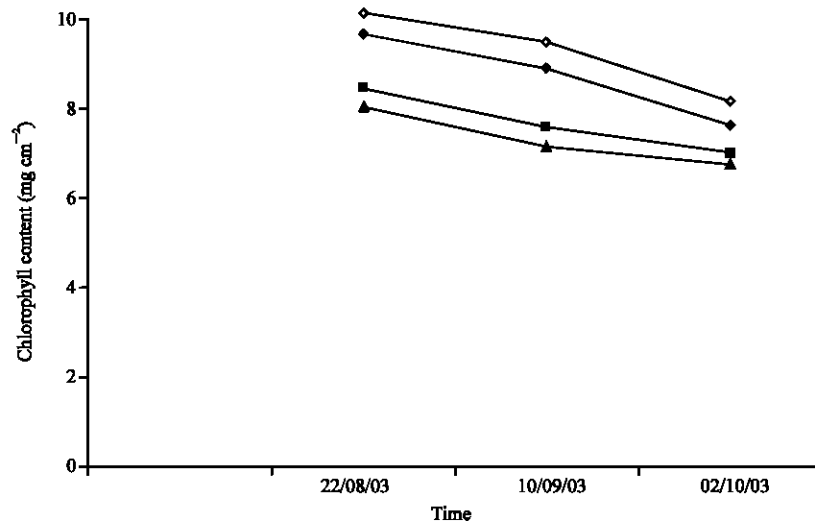


Fig. 3: Chlorophyll concentration of cotton cultivar Campo when influenced by Bermudagrass at 0 (▲), 21 (■), 42 (◆) DAS and control (○)

Decrease in Fv/Fm values was scaling and depended on time of rhizome planting. Inferior Fv/Fm values were measured in cotton plants when interfered with Bermudagrass at 0 DAS, followed in ascending order by 21, 42 DAS and control. For cultivar Campo, Fv/Fm ranged between 0.58-0.68 in 0 DAS, 0.62-0.72 in 21 DAS, 0.7-0.75 in 42 DAS and 0.65-0.77 in control (Fig. 5). For cultivar Millenium, Fv/Fm ranged between 0.65-0.75 in 0 DAS, 0.66-0.75 in 21 DAS, 0.65-0.79 in 42 DAS and 0.66-0.77 in control (Fig. 6).

These results show that cotton is highly susceptible to Bermudagrass. Stomatal resistance was increased when cotton plants interfered with Bermudagrass, even at a delayed rhizome planting at 42 DAS. It has to be noted that although stomatal aperture was significantly reduced, no other symptoms such as leaf curling or rolling to reduce the area exposed to sunlight, or movement of leaves to parallel the sun rays or shedding of older leaves were observed to implicate water stress status of cotton plants. Chlorophyll content on cotton leaves was

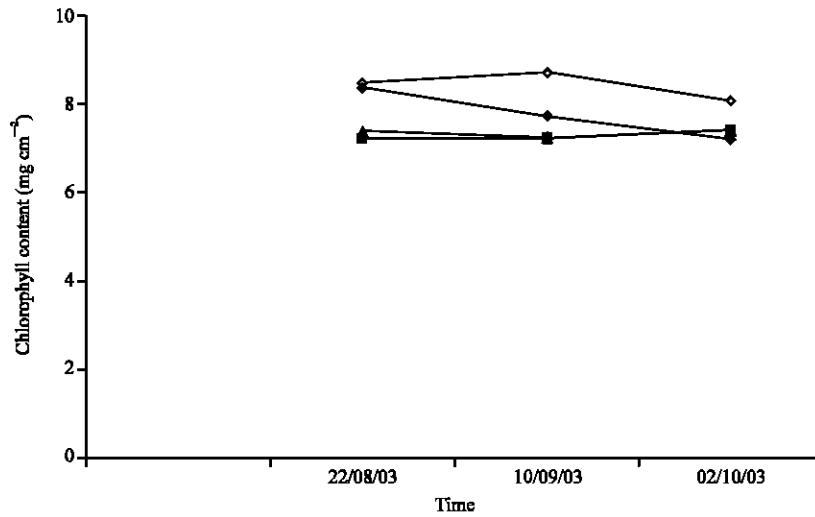


Fig. 4: Chlorophyll concentration of cotton cultivar Millenium when influenced by Bermudagrass at 0 (▲), 21 (■), 42 (◆) DAS and control (◇)

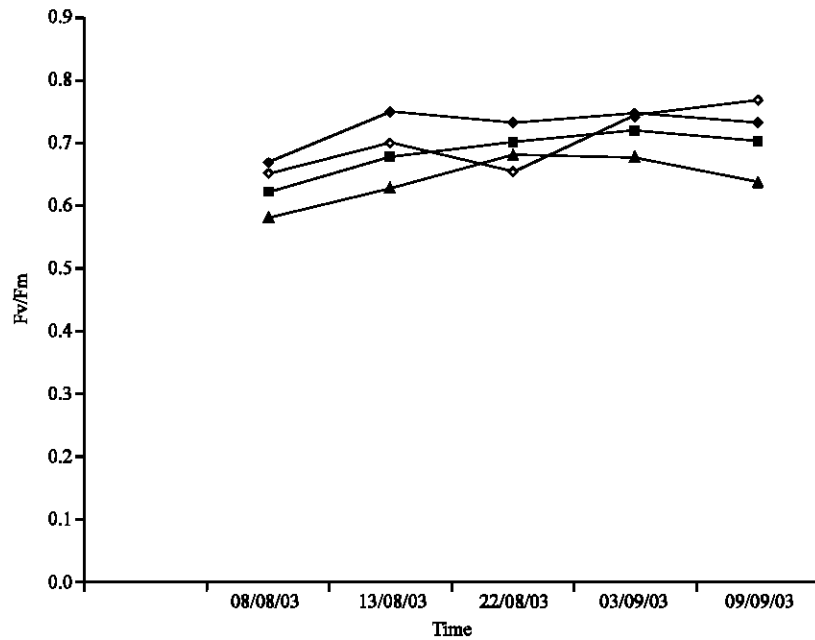


Fig. 5: Chlorophyll fluorescence of cotton cultivar Campo when influenced by Bermudagrass at 0 (▲), 21 (■), 42 (◆) DAS and control (◇)

significantly reduced when affected by Bermudagrass. Such reduction was observed even when rhizomes were planted at 42 DAS ( $p = 0.0805$ ; this value is significant at 10% level of significance). It remains to be studied if this symptom can be attributed to synthesis inhibition or degradation stimulation of chlorophyll or possibly due to a combination of two. Similarly to chlorophyll content, fluorescence function was also significantly decreased

when weed rhizomes were planted adjacently to cotton at 0 DAS ( $p = 0.0029$ ). This symptom was less pronounced with a delayed planting of Bermudagrass near cotton (at 21 and 42 DAS).

Chlorophyll content and Fv/Fm values have also been used to measure stress of plants. Meloni *et al.* (2003) observed no alterations in Fv/Fm values at two cotton cultivars when imposed them in salt stress whereas

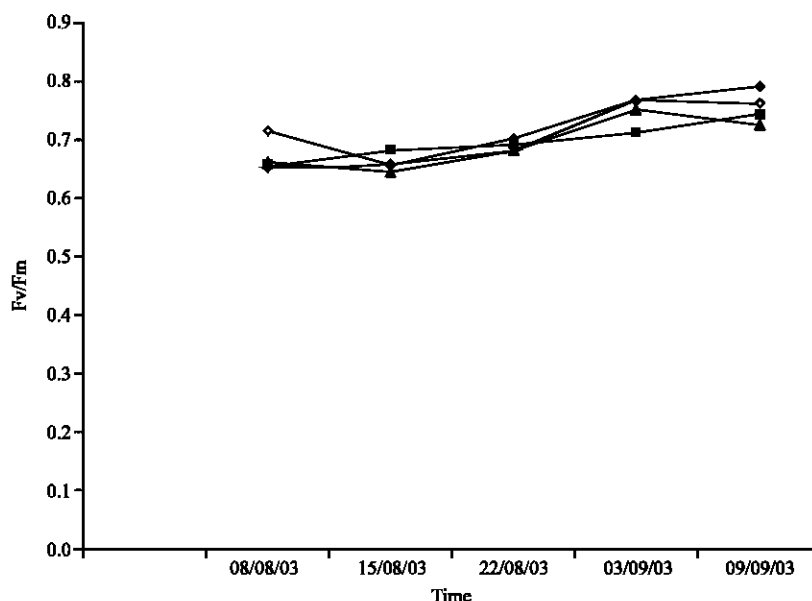


Fig. 6: Chlorophyll fluorescence of cotton cultivar Millenium when influenced by Bermudagrass at 0 (▲), 21 (■), 42 (◆) DAS and control (◇)

total chlorophyll content was reduced by 35%, but in only one of the cotton cultivars used. Similarly, Santos (2004) observed that PS II in sunflower leaves was not affected after sunflower was exposed in salt stress, whereas chlorophyll content was reduced even at the lowest salt concentration. PS II function and chlorophyll content have also been studied in plants under water deficiency conditions. Colom and Vazzana (2003) imposed *Eragrostis curvula* plants in water stress and found that PS II function and total chlorophyll content were adversely influenced whereas Souza *et al.* (2004) found that PS II function in cowpeas (*Vigna unguiculata*) was reduced only after five days of water stress. Flagella *et al.* (1996) observed that PS II function in wheat plants was severely damaged only in serious water stress and only for the susceptible variety used in the experiment.

Agronomic characteristics measured in cotton plants were adversely affected when influenced by Bermudagrass. Maximum reduction was observed at 0 DAS. Maximum reduction was 41.7% for stem height, 62.0% for root dry matter, 61.9% for stem dry matter and 34.7% for stem diameter (Table 2).

Millenium yielded superior stem height ( $p = 0.0002$ ) and stem diameter ( $p = 0.0093$ ); Campo yielded superior root dry matter ( $p = 0.0117$ ). No differences were found for stem dry matter ( $p = 0.4864$ ). All agronomic characteristics were significantly increased ( $p < 0.0001$ ) in the second year of the experiment. Interaction year of planting  $\times$  cultivar was significant ( $p = 0.0222$ ). No other interaction between factors was found significant. Results show that growth

Table 2: Means and standard error of the mean ( $s_e$ ), of agronomic characteristics for two cotton cultivars (Campo and Millenium) when influenced by Bermudagrass in a two-year experiment

Treatments	Agronomic characteristics			
	Stem height (cm)	Root DM (g)	Stem DM (g)	Stem diameter (cm)
Control	24.60±0.88 <sup>a*</sup>	2.34±0.15 <sup>a</sup>	3.65±0.22 <sup>a</sup>	0.72±0.015 <sup>a</sup>
0 DAS	14.35±0.87 <sup>b</sup>	0.89±0.12 <sup>b</sup>	1.39±0.22 <sup>c</sup>	0.47±0.015 <sup>c</sup>
21 DAS	18.23±0.89 <sup>b</sup>	1.28±0.15 <sup>b</sup>	2.10±0.22 <sup>b</sup>	0.40±0.015 <sup>c</sup>
42 DAS	21.75±0.90 <sup>a</sup>	1.99±0.20 <sup>a</sup>	3.23±0.23 <sup>a</sup>	0.65±0.015 <sup>b</sup>

\*: Within columns, means followed by the same letter(s) are not significantly different at  $p = 0.05$

inhibition was more severe when weed was early planted at 0 DAS which is in agreement with Vencill *et al.* (1992, 1993). In particular, root system of cotton remained totally undeveloped and not efficient to contribute to proper plant nutrition and growth. Similar symptoms of Bermudagrass on vine roots were referred by Mez (1961).

Soil analysis did not reveal any differences in nutrient content, pH, EC or organic matter between treatments which could possibly affect cotton growth.

In conclusion, findings of this study indicate that stomatal resistance, chlorophyll content and chlorophyll fluorescence can be considered as reliable indicators to measure stress on cotton plants induced by interference with Bermudagrass.

#### ACKNOWLEDGMENTS

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