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Allelopathic Susceptibility of Cotton to Bermudagrass

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Abstract: The aim of the study was to investigate the influence of bermudagrass on growth components, physiology parameters (stomatal aperture, leaf transpiration, carbon dioxide intake, leaf temperature, Fv/Fm values and net assimilation rate) and concentration of important nutrients (N, P, K, Mg, Ca) in leaves and roots of cotton. Cotton seedlings and bermudagrass plantlets were growing adjacently in a Hoagland's nutrient solution of ½ strength, which was continuously circulated. Under the influence of bermudagrass, cotton suffered significant reduction of stomatal aperture, leaf transpiration, CO_2 intake, Fv/Fm values, net assimilation rate and significant increase in leaf temperature. No differences were recorded between control and treatment in nutrient concentration in leaves. On the contrary, in cotton roots due to the influence of bermudagrass, Mg was increased by 29.2, P by 63.2 and Ca by 297.4%. Consequently the growth of plants in treatment was significantly inferior than control. Reduction was recorded at 54.6% for stem dry matter, at 35.5% for stem diameter, at 55.3% for leaves dry matter and 46.2% for root dry matter. Growth inhibition and deterioration of photosynthesis components (specifically Fv/Fm values) indicate that cotton suffered serious allelopathic stress due to the weed influence. Stress effect was pronounced although plants were growing in the nutrient solution, were no adverse factor to limit growth was observed. The allelopathic effect was exhibited by the weed, although it was not stressed in the nutrient solution. Dilution of allelochemicals in a high volume solution didn't reduce activity of allelochemicals.

Key words: Weed interference, stress, growth inhibition, photosynthesis reduction, Fv/Fm, nutrient solution

INTRODUCTION

Despite the advances in the understanding of weed biology, the sophisticated control methods and the improvement of the equipment used, weeds significantly reduce farmers income, by reducing crop yield and quality of products.

Weeds have been proved higher competitors than crops, because of superior morphological characteristics that develop in roots and stems, as well as higher growth rate that enable them to exploit better resources and dominate in the cultivated fields (Dunbabin, 2007). Today the concept of competition between plant species has been supplemented with that of plant allelopathy. Age and plant stress are the key factors that dictate allelochemical production in a plant. Decline of allelochemical contents in living plants with increasing age of plants is a general case, while periodic production may be a special case (An *et al*., 2003). Controversial experimental results, difficulties in separation of allelopathy from resource competition and coexistence of allelopathy and resource competition in the natural environment make scientists skeptics to distinguish allelopathy from resource competition. Weißhuhn and Prati (2009) refer allelopathy as a controversially debated phenomenon for years, whereas Inderjit (2006) concluded that inhibitory effects on growth could be due to number of effects that could be misconstrued as allelopathic effects. Also, Hong *et al*. (2003) found that plant parts of radish showed different response to the influence of *Clerodendrum infortunatum*. The fact is that plants compete for resources (such as nutrients) but they also produce and release in their environment chemical compounds that usually cause detrimental effects to their neighbors. From this point of view, resource competition and allelopathy must be both considered when studding plant interference. Further, due to the complex and not well understood interference relations between plants,

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unreasonably high standards of evidence for establishing of allelopathy are demanded from some researchers, standards that are not demanded for other plant-plant interactions such as resource competition (Inderjit and Weiner, 2001).

A wide range of experimental methods is needed for the understanding the interactions between plants, but also the influence of donor plants on the environment that subsequently can influence the target plants. Petri dishes bioassays (Lee *et al*., 2002), pot experiments, field surveys and growth of plants in non-soil substrates (Hao *et al*., 2007) are among the methods that are used in order to describe better the concept of allelopathy (Rafiqul Hoque *et al*., 2003; Nasr and Mansour, 2005; Yu *et al*., 2003; Travlos *et al*., 2008). Different methods can be proven complementary as allelopathy requires multifaceted approach because allelochemicals can act in different sites of plant metabolism. Growth components such as plant height, stem diameter or leaf expansion were first used to measure the effects of plant species to their neighbors in order to dominate in their site. In most of circumstances, inhibitory effects are observed between plants that were interfered. This is probably the reason that allelopathy is associated with adverse effects in growth development of plants. In order to identify the effects of allelochemicals in plant metabolism, specific biochemical processes were studied. Bouchagier *et al*. (2008), studied the allelopathic influence of bermudagrass on cotton by measuring physiochemical characteristics such as stomata conductance, chlorophyll concentration and chlorophyll fluorescence. They found that when growing in soil, bermudagrass significantly reduced all growth parameters (stem height, stem diameter, number of expanded leaves) but also caused significant reduction in chlorophyll concentration and chlorophyll fluorescence in cotton leaves.

The aim of this research was to study the allelopathic interference between bermudagrass and cotton in a hydroponic system. Photosynthesis components (stomata aperture, leaf transpiration, CO_2 absorption, leaf temperature, net assimilation rate and Fv/Fm values) were examined as reliable indicators for the description of stress status of cotton plants due to bermudagrass influence.

MATERIALS AND METHODS

The experiment was conducted in two consecutive years (from 25 May to 18 August 2002 and from 26 May to 2 August 2003) in the research site (outdoors) of the Agricultural University of Athens, Greece. Cotton seeds (cultivars Campo and Millenium) were sown in sand, in cylindrical tubes (height 30 cm, diameter 20 cm). Also root cuttings of *C. dactylon* (5-6 nodes length) were planted in sand to initiate rooting. No fertiliser was added during seedlings and plantlets growth in tubes. Bermudagrass plantlets and cotton seedlings were transferred to vessels 25 Days After Sowing (DAS) when the first leaf of cotton was expanded (stage 11 BBCH scale) in a ½ strength Hoagland's solution. Nutrient solution was continuously circulated. At the end of each day, vessels were refilled in order to replace water lost by evapotranspiration. During plant growth, pH of nutrient solution was recorded at 8 and Electrical Conductivity (EC) at 2 m S cm^{-1}.

In the first year stomatal resistance of cotton plants was measured with a AP A4 porometer. Resistance was measured 12 times from 2-18 August 2002 in regular intevals (1-2 days) on the adaxial surface of fully expanded intact functional leaves. Each time, resistance was measured in 15 plants (1 leaf/plant) between 12.00 to 13.00 h.

In the second year stomatal conductance (the reciprocal of stomatal resistance), leaf transpiration, CO_2 absorption, net assimilation rate and leaf temperature in cotton leaves were measured with a CIRAS-1 instrument. Fluorescence function was measured by means of Fv/Fm values (Fv: variable fluorescence, Fm: maximum fluorescence) with a Fim 1500 instrument. Measurements were taken between 11.00 to 13.00 h from the adaxial leaf surface of fully expanded and intact functional leaves. For stomatal conductance, leaf transpiration, CO_2 absorption, net assimilation rate and leaf temperature, 7 measurements were taken from 3-23 July 2003 in regular (3 days) intervals. For Fv/Fm values in leaves, 7 measurements were taken in regular (1-2 days intervals) from 14-23 July 2003.

At the end of the experiment, cotton plants were harvested and stem diameter and number of buds were recorded. Then stems, leaves and roots were oven dried to a constant weight and stem dry matter, leaves dry matter and root dry matter were measured. Also shoot: root ratio was calculated. Cotton plants were further processed to measure N, P, K, Ca and Mg concentration in roots and leaves according to Jones and Case (1990).

The experiment followed the Completely Randomised Design and was analysed as factorial with two factors: (1) Cotton cultivar (2 levels, Campo and Millenium), (2) Treatments [2 levels, control (weed free) and treatment (weed influence)]. Sample size for agronomic characteristics and nutrient concentration was 64 plants (2 cultivars×2 treatments/cultivar×16 plants/treatment/cultivar). Treatments were compared with Student *t*-test with one df (degree of freedom) for cultivars and one df for treatments.

For photosynthetic characteristics statistical analysis was made according to the Repeated Measurements Design, using F test with one df for cultivars and one df for treatments. For stomatal resistance, stomatal conductance, leaf transpiration, CO_2 absorption, leaf temperature and net assimilation rate, sample size was 48 plants (2 cultivars×2 treatments/cultivar×12 plants/treatment/cultivar). For Fv/Fm values, sample size was 56 plants (2 cultivars×2 treatments/cultivar×14 plants/treatment/cultivar).

All comparisons were made at 5% level of significance. Prior to statistical analysis, data were tested for homogeneity of variances and for normal distribution by Shapiro-Wilk test. Where necessary, Box-Cox transformation of data was used. Analysis was performed with the statistical software JMP (2003).

RESULTS AND DISCUSSION

Weeds still represent a major causal factor for the reduction of crop growth and productivity. They compete with crops for natural resources such as nutrients, water or even space. Effective weed control remains the demand for production systems in both developed and developing countries. Research efforts are dedicated in order to understand the mechanisms developed by weeds that make them able to invade in new sites, expand and dominate in the agricultural fields. Plant allelopathy is a concept that is currently supported by increasing number of agronomists and denotes the production of specific biomolecules (allelochemicals) by one plant that can induce suffering in, or give benefit to, another plant. However it is most commonly used in the former sense, an interaction in which one plant causes suffering to another plant (Babula *et al*., 2009).

Due to inhibitory effects on growth of target species, allelopathy is considered as a promising weed management tool. Chemical substance secreted from plant roots are found to inhibit seed germination of target species (Turk and Tawaha, 2003; Djurdjevic *et al*., 2004; Sharma and Gupta, 2007). Allelochemicals have been found to reduce growth development and injure physiology characteristics (Sodaeizadeh *et al*., 2010). Romero-Romero *et al*. (2005) compared the allelopathic stress of *Sicyos deppei* on *Lycopersicon esculentum* roots with water stress. Kaur *et al*. (2005) showed that root cells of mustard grown in soil treated with benzoic acid were disorganized, distorted and deformed compared to control. Solidification of cell walls, increased POD activity and lignin content and finally premature cessation of root growth of soybean roots was observed after the application of p-coumaric acid (Zanardo *et al*., 2009). Ocimenones, that are classified as terpenoids delayed and inhibited the germination of cohabitant species of *Tagetes minuta* (López *et al*., 2009).

The concept of allelopathy is also extended to explain the invasion plants ability to establish in their naturalized range than in their native range (Clements *et al*., 2004). Surprisingly, allelopathy appears not to be the key factor for the domination of invasive plants in their new habitats (Renne *et al*., 2004; Inderjit *et al*., 2006).

Allelopathy has been proved an attractive topic for the development of mathematical models to study the phenomenon of such a chemical defense mechanism (Dubey *et al*., 2010). Mathematical models have also been expanded in order to describe autotoxicity (Sinkkonen, 2007).

In our research we studied the allelopathic effects of bermudagrass to cotton. Previous studies revealed the adverse effects of bermudagrass on vegetative growth and physiological characteristics of cotton when plants were growing in soil (Bouchagier and Efthimiadis, 2003). In our experiments cotton plants also remained stunted when interfered with bermudagrass in a nutrient solution. In both years of experimentation, stomatal aperture of cotton plants was significantly reduced ($p<0.0001$) when influenced by bermudagrass (Fig. 1).

Fv/Fm values in cotton leaves confirmed the significant adverse influence ($p<0.0001$) of bermudagrass to cotton (Fig. 2, 3). Also, measurements of leaf transpiration, CO_2 absorption and net assimilation rate in cotton leaves were inferior in treatment than control. Leaf temperature in cotton leaves was measured significantly increased in treatment than control. Differences between cotton cultivars on the relative sensitivity against bermudagrass were observed for leaf transpiration ($p = 0.0305$), net assimilation rate ($p = 0.0445$) and Fv/Fm ($p = 0.0012$). Cotton growth was clearly influenced and

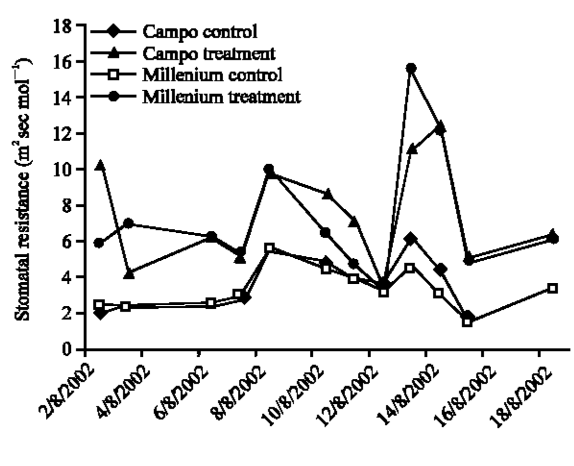

Fig. 1: Stomatal resistance of cotton

Table 1: Means and standard errors of the mean ($S_{\bar{x}}$) for two cotton cultivars, for several agronomic characteristics of cotton when interfered with bermudagrass in a nutrient solution

	Agronomic characteristics					
Treatments	Stem DM (g)	Stem diameter (cm)	Number of buds	Leaves DM (g)	Root DM (g)	Shoot:root ratio
Control	3.13±0.19[a]*	0.76±0.02[a]	14.6±0.4[a]	3.78±0.23[a]	1.71±0.12[a]	4.39±0.32[a]
Treatment	1.42±0.19[b]	0.49±0.02[b]	10.4±0.4[b]	1.69±0.23[b]	0.92±0.12[b]	4.13±0.32[a]
Reduction	54.60%	35.50%	28.80%	55.30%	46.20%	5.90%

Within columns, means followed by the same letter are not significantly different at p = 0.05 probability level

Table 2: (%) nutrient concentration (mean±standard error of the mean) of cotton roots when plants were growing in a nutrient solution

	Nutrients				
Treatments	N	K	Mg	P	Ca
Control	4.38±0.078[a]	4.48±0.16[a]	0.24±0.01[b]	0.19±0.016[b]	0.78±0.31[b]
Treatment	4.31±0.072[a]	4.54±0.16[a]	0.31±0.01[a]	0.31±0.016[a]	3.10±0.72[a]
Reduction	1.60%	-1.30%	-29.20%	-63.20%	-297.40%

Within columns, means followed by the same letter are not significantly different at p = 0.05 probability level

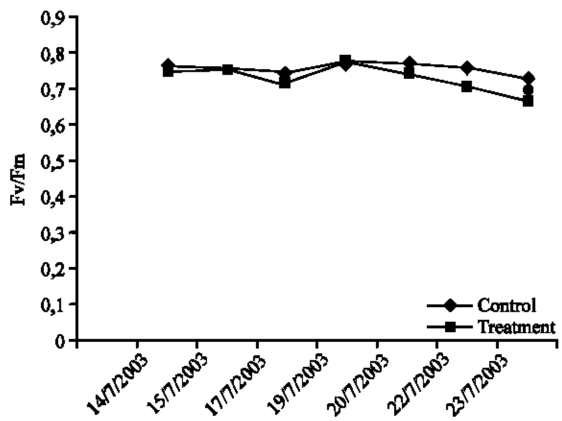

Fig. 2: Fv/Fm values of cotton (cultivar Campo)

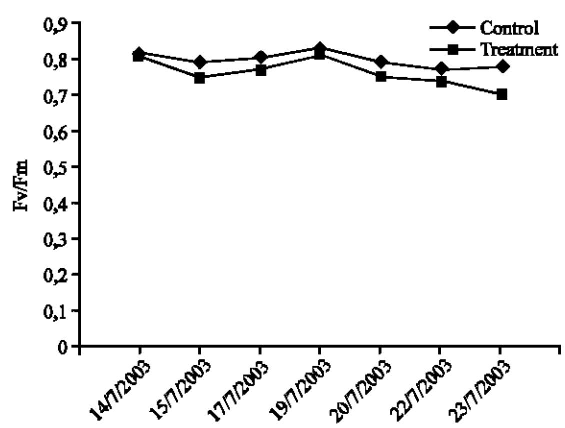

Fig. 3: Fv/Fm values of cotton (cultivar Millenium)

agronomic characteristics were suppressed due to bermudagrass. Leaves dry matter and stem dry matter were mostly reduced by 55.3 and 54.6% correspondingly. Shoot:root dry matter exhibited a small reduction (5.9%). This is in agreement with results previously obtained when plants were growing in soil (Hopkins, 1999) (Table 1).

No differences were observed between control and treatment in cotton leaves for N, K, Mg, P and Ca (data not shown). In cotton roots Mg, P and Ca in treatment were measured in significantly higher concentrations than control (Mg by 29.2, P by 63.2 and Ca by 297.4%). No differences were observed for N and K (Table 2).

Accumulation of Mg, P and Ca in cotton roots may have been achieved through a modification of cotton metabolism. Possibly phosphorus, magnesium and calcium accumulation in roots, either by selected increased uptake or redistribution from the rest cotton plant as such has been referred to occur when plants are influenced by phenolic acids (Jung *et al*., 2004), might be the induction of further stabilization of cell membranes as these have been identified a possible target of allelochemicals (Yu *et al*., 2003).

During the experiment plants were aplenty nutrified. This was assured by employing a large volume of nutrient solution (Taiz and Zeiger, 1991). Not any resource limitation (for water, light, CO_2 etc.) was identified and not any deficiency symptoms were developed in plants. Thus it is possible that inferior growth, adverse effects on physiological characteristics and alterations in nutrient absorption, would be attributed to the activity of allelochemicals produced and released to the solution by the roots of bermudagrass. Allelochemicals exhibited strong activity although they were diluted in high volume of nutrient solution. In fact dilution in the nutrient solution may have enhanced their effect, as any possible reduction of theirs activity due to biotic or abiotic factors (Dakshini *et al*., 1999), or due to absorption on soil particles (Ito *et al*., 1998) was avoided.

Bermudagrass exhibited a strong allelopathic effect to cotton although plants didn't suffer any kind of stress. Contrary to Reigose *et al.* (1999) who stated that plant allelopathy is developed only by stressed plants, it appears that production of allelochemicals is not necessarily connected with adverse conditions caused by biotic or abiotic factors, but it may also happen even a donor species has fulfilled its requirements for resources. Moreover, bermudagrass appeared to influence multiple sites of cotton metabolism.

In this study hydroponics was employed to study interference between plants and separate if possible, resource competition from allelopathy. Although plants were developed in artificial conditions, significant differences on growth, physiology and nutrient absorption, were developed due to plant interference. Hydroponics is proposed as a reliable, convenient and time saving method which also can assure removal of possible variability in soil conditions or population density of weeds.

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