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IMPACT OF SHADING AND CHLORMEQUAT CHLORIDE ON LANTANA SPECIFIC LEAF AREA AND MINERAL CONTENT

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ABSTRACT

The creation of high quality plants and their maintenance depends, primarily, on proper fertilization, deriving mainly from the knowledge of their nutrient requirements (NR). Spray-applied chlormequat chloride (CCC), at the concentrations of 3000 and 6000 mg L^{-1}, has been reported to contribute to the production of attractively flowering *Lantana camara* L. subsp. *camara* (lantana) plants. However, no information is available about the NR of these plants. The effects of the aforementioned CCC concentrations plus 0 mg L^{-1} (control) on specific leaf area (SLA) and leaf potassium (K), phosphorus (P), calcium (Ca), iron (Fe) and manganese (Mn) contents of lantanas were examined under the 0% and 66% shading levels. Chlormequat chloride (3000 and 6000 mg L^{-1}) decreased, generally, SLA of lantanas compared to control, at both shadings. Increased SLA values were estimated with increased shading. Phosphorus content increased with the increased CCC concentrations at 66% shading level. Phosphorus and Fe contents increased while Ca and Mn decreased with the increasing shading. The results of our study may contribute to the planning of a fertilization program for CCC-treated *Lantana* plants, a promising genus in landscaping in many countries around the world.

Key words: Chlormequat chloride; *Lantana camara* L.; nutrition; onium-type regulator; specific leaf area; photosynthetic photon flux.

INTRODUCTION

Lantana camara L. is a plant species with great ornamental potential, due to gathering of many desirable features, such as attractive colored flowers, (Ramírez-Hernández *et al.* 2012) and continuous bloom of flowering during the hot period of the year (Ruter 1996). Also, *Lantana* is an important medical plant with antimicrobial properties (Naz and Bano 2013). This genus may be used in flower beds, edgings or along walls or fences (Lorenzi and Mello-Filho 2001) and it is used widely in landscape design in several regions of the world like Mediterranean countries (Matsoukis and Kamoutsis 2003; Garibaldi *et al.* 2008) and Asia (Naqvi *et al.* 2013).

Chlormequat chloride, an onium-type regulator, chemically named (2-chloroethyl) trimethylammonium chloride, inhibits, generally, gibberellin and sterol biosynthesis (Rademacher 1991) and causes enhanced or retarded plant growth even in the same plant species, e.g. *Dahlia pinnata* L. (Hossain *et al.* 1999) as well as decreased leaf area (LA) on lantanas compared to control plants (Matsoukis *et al.* 2004). This regulator may contribute to the creation of attractive lantana fences by increasing the number of flower heads, especially at the concentrations of 3000 and 6000 mg L^{-1} (Matsoukis and Chronopoulou-Sereli 2005) but no information is available from literature about substantial variables of growth of CCC-treated lantanas such as nutrient requirements (NR) and specific leaf area (SLA).

Nutrient requirements may be assessed satisfactorily by leaf tissue analysis (Van den Driessche 1974), a widely used quantitative diagnostic technique (Lucena 1997; Romero *et al.* 2014). The knowledge of NR is essential for the establishment of fertilization programs which are necessary to maintain as much as possible a high quality flowering CCC-treated lantana. Specific leaf area, defined as the projected leaf area per unit leaf dry matter (DM) (Evans and Poorter 2001), may be evaluated as an informative summary variable of plant metabolism as it has been related to several relative variables, e.g. foliar nutrient contents (Burns 2004).

Many growers alter light availability, by using shading materials above cultivated plants, especially in cases of undesirably high values of solar radiation during summer period, aiming to protect their plants by preventing the direct effect of solar radiation and the resulting quick increase of air temperature (Chronopoulou-Sereli and Flocas 2010). The necessity for carrying out the present work resulted from the test of the hupothesis of the different physiological and nutritional response of *Lantana* plants to different CCC concentrations and environments. Therefore, the objective of our research was to investigate the effects of CCC on physiological (SLA) and nutritional (K, P, Ca, Fe and Mn contents) characteristics of *Lantana* plants. Additionally, attempting to find possible relationships

between environmental conditions and production of high quality plants, the impact of different light regimes was also tested. The results of this study will provide new insights on the effective development of an appropriate fertilization program for CCC-treated *Lantana* plants under changing environments.

MATERIALS AND METHODS

Lantana plants derived from 16 to 18 cm mid-stem cuttings, grew in a glasshouse in Attica ($37^0 48' 20'' N$, $23^0 57' 48'' E$), Greece. Details of growth and experimental conditions have been reported in Matsoukis and Chronopoulou-Sereli (2005). Chlormequat chloride (Affix 60 A.S. 60% w/v, Chemische produkten, Gesellschaft, Germany) was sprayed (24 June) on foliage of lantana plants at 0 (control), 3000 and 6000 mg L^{-1} using 0.25 L of solution per plant. At the same time, lantanas were placed to two plots where different levels of photosynthetic photon flux density (PPFD) were applied. Thus, there was a plot covered with black, dense woven net (66% shading level), model 201 (Manioudaki Bros S.A. knitting factory, Greece). A non shaded plot (0% shading level) was also included. The two PPFD regimes for 0% and 66% shadings provided average daily light quantities of 27.8 and 9.4 mol m^{-2} d^{-1}, respectively. Mean daily maximum and minimum temperatures ranged from 27.2 °C (66% shading) to 30.4 °C (0% shading) and from 17.1 °C (66% shading) to 17.5 °C (non shaded plot), respectively. The mean daily relative humidity was higher (70%) at 66% shading without a difference more than 5.3% between the shading levels. Plants were irrigated as needed, with water containing K at 0.05 meq L^{-1}, Ca at 2.50 meq L^{-1}, magnesium (Mg) at 3.0 meq L^{-1}, bicarbonate (HCO_3) at 5.0 meq L^{-1}, sodium (Na) at 0.50 meq L^{-1} and chlorine (Cl) at 0.3 meq L^{-1} with a pH of 6.8 and electrical conductivity of 463 μS cm^{-1}.

At the end of the experimental period (23rd November), 5 months after the application of CCC, 512 leaves, with no signs of senescence, from each CCC treatment (consisted of 8 plant-replicates) were collected from each shading level. The collection was made from the second to ninth node of the four highest shoots of each plant. Leaf area of each leaf (excluding the petiole) was measured with a leaf area meter (LI-3000 A, LICOR, Lincoln, USA). Leaves (totally 3072) were then oven-dried in paper bags at 65 °C for 60 h till constant dry weight, giving a DM. Specific leaf area was calculated as LA divided by DM. Sub-samples used for the determination of SLA were also used for the determination of K, P, Ca, Fe and Mn. The dry leaf samples were ground (40 mesh sieve) to a fine powder. For the analysis, 0.5 g of ground dry leaf samples was placed in porcelain crucibles and ashed in a muffle furnace (type 48000, Model F 48020-26, Barnstead/Thermolyne, Dubuque, USA) at temperature of 550 °C for 4 h. The ashes were subjected to wet digestion in 15.8 M HNO_3. Potassium, Ca, Fe and Mn were analyzed with atomic absorption spectrometry (Varian SpectrAA 300, Varian Inc., Palo Alto, USA) (Jones and Case 1990; Gasparatos *et al*. 2011) while P was determined colorimetrically (Roussos *et al*. 2007). All analyses were made twice and an average value was calculated for each element and plant-replicate (8 samples-replicates/plant) in each regulator treatment.

The experiment was carried out according to the two factor completely randomized design. The first factor had two examined shading levels and the second factor three levels, each corresponding to each applied CCC concentration (including controls). For the examined variables (SLA and P, K, Ca, Fe and Mn contents), the calculated means were used for analysis of variance (ANOVA). The two (out of eight) extreme means of each CCC treatment were excluded from data before analysis. For the figure, standard errors presented at its right side, were calculated from the residual variances of the analysis in accordance with previous works (Roussos *et al*. 2007; Roussos 2013) and they were used for the comparison of means (Roussos 2001), where appropriate from the ANOVA. Statistics was performed using SPSS version 8.0 for Windows and MS Excel 2007. Results were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Chlormequat chloride concentration and shading level showed significant effects on SLA, P and Fe contents of lantana plants while Ca and Mn contents were affected only by shading level (Table 1). Neither shading, nor CCC concentration affected K content which showed no pattern of change ranging from 6.60 to 9.20 mg g^{-1} DM. Specific leaf area in CCC-treated plants (3000 and 6000 mg L^{-1}) was generally lower (Fig. 1a) than the respective value of control plants (0 mg L^{-1}), due to the greater LA reduction than the leaf DM reduction, irrespective of shading level. The aforementioned LA reduction in lantanas may be due to inhibition of both cell division and cell elongation after the treatments with CCC in accordance with Lodeta *et al.* (2010) who reported a similar hypothesis for CCC-treated *Euphorbia pulcherrima* cv. Christmas Feelings plants (at concentrations of 1500 and 3000 mg L^{-1}). Altintas (2011) reported that CCC as a spray or drench at lower concentrations (2000 mg L^{-1}) than those of our study, did not affect SLA of *Lycopersicon esculentum* Mill. cv. Maya F_1. Lantana SLA values under 66% shading were always higher ($P < 0.05$) in relation to 0% shading considering the same CCC concentration (Fig. 1a). These SLA increases were ranged from 22% (CCC at 6000 mg L^{-1}) to more than two times this percentage (control) and they were due to the greater increases of LA than DM. These LA increases may be attributed partially to the

more favorable growth conditions at 66% shading, due to logically reduced evaporation resulting from the shade net and the subsequent decreased air temperature values, compared to 0% shading, in agreement with Farque *et al.* (2001) for *Quercus petraea* (Matt.) Liebl. plants grown at 18% sunlight (equivalent to 82% shading). The aforementioned LA increases may be considered as a part of the *Lantana camara* L. subsp. *camara* behaviour to increase its competitive ability under 66% shading (Matsoukis *et al.* 2014). Increased SLA values have been reported in *Clematis manshurica* Rupr. with a progressive increase of shading from 0% to 90% (Wang *et al.* 2010). In addition, increasing shading from 30% to 70% caused steadily higher values of SLA, with or without significant differences from each other, in *Adhatoda beddomei* C.B. Clarke plants (Neerakal *et al.* 2005). Also, paclobutrazol (PBZ), a growth regulator of the triazole class, induced increased SLA values at lantanas grown at 66% shading compared to 0% shading (Matsoukis *et al.* 2007; 2014).

Table 1. Analysis of variance for effects of shading level (SL) (0% and 66%) and chlormequat chloride (CCC) concentration (0, 3000 and 6000 mg L^{-1}) on specific leaf area (SLA) and leaf potassium (K), phosphorus (P), calcium (Ca), iron (Fe) and manganium (Mn) content, on dry matter (DM) basis, of *Lantana camara* L. subsp. *camara* plant.

	SLA ($cm^2\ g^{-1}$ DM)	K (mg g^{-1} DM)	P (μg g^{-1} DM)	Ca (mg g^{-1} DM)	Fe (μg g^{-1} DM)	Mn (μg g^{-1} DM)
	Variance ratio					
SL	29.59^{***}	1.96 ns	12.40^{***}	21.16^{***}	26.76^{***}	15.70^{***}
CCC concentration	3.44^{*}	3.02 ns	18.30^{***}	1.51 ns	5.89^{**}	2.68 ns
SL × CCC concentration	1.69 ns	1.29 ns	1.07 ns	3.19 ns	3.14 ns	0.98 ns

***, **, *: significant at $P \leq 0.001$, ≤ 0.01 and ≤ 0.05, respectively. ns: not significant ($P > 0.05$).

The contents of P and Fe increased with CCC at 3000 mg L^{-1} ($P < 0.05$) compared to 0 mg L^{-1}, followed by a reduction at the highest CCC concentration (significant only in the case of Fe in relation to smaller concentrations), at 0% shading level (Fig. 1b, d). At 66% shading, P content increased continually as the concentration of CCC increased (Fig. 1b) while Fe exhibited slight increases (Fig. 1d). The examined mineral elements in lantana plants showed different patterns of change with the increasing concentrations of CCC, independently of shading level. Therefore, further investigation is required to elucidate this topic. Matsoukis *et al.* (2009; 2014) reported increased content of P in PBZ-treated lantanas at the examined light environments.

Phosphorus and Fe contents increased, while Ca and Mn decreased, as shading increased ($P < 0.05$), when examining the same concentration of the regulator including controls (Fig. 1b, c, d, e). The decreased uptake of P and Fe from lantanas at 0% shading, in relation to 66% shading, may be affected by the possible temporary dryness of the substrate surface at the non shaded plot during the hot days just before irrigation. A similar hypothesis was reported by Matsoukis *et al.* (2014) for N, P, K and Mg contents of control lantana plants grown at a full light environment. The decreased Mn content of control plants at 66% shading compared to 0% shading, may be attributed, in part, to antagonistic effects among trace elements, like Mn and major elements like P, which increased at 66% shading as mentioned before (Kabata-Pendias and Pendias 2001). There are no comparable studies from literature on the effect of shading on mineral content of CCC-treated plants. Increased P and K contents have been reported in the leaves of PBZ-treated lantana at 66% shading compared to 0% shading (Matsoukis *et al.* 2009; 2014). All experimenral plants, as confirmed by visual observations, were free of nutrient insufficiency symptoms while to our knowledge, no foliar nutrient sufficiency ranges are available from literature on *L. camara* plants.

In conclusion, decreased SLA values of lantana were estimated, generally, after CCC applications (3000 and 6000 mg L^{-1}), irrespective of shading and increased SLA values were estimated with increased shading. As CCC concentration increased at the low light environment (66% shading), so did P content. The lowering of light intensity from the non shaded to shaded plot caused increased P and Fe and decreased Ca and Mn contents, indicating thus, that the low light intensity (66% shading) seems to affect the nutrition of lantana plants with regard to the examined mineral elements. Our study could add knowledge towards the creation of a fertilization program for CCC-treated *Lantana* plants. In the future, research may focus on SLA and mineral content of other *Lantana* species (and/or subspecies) testing similar or different concentrations of CCC and PPFD conditions.

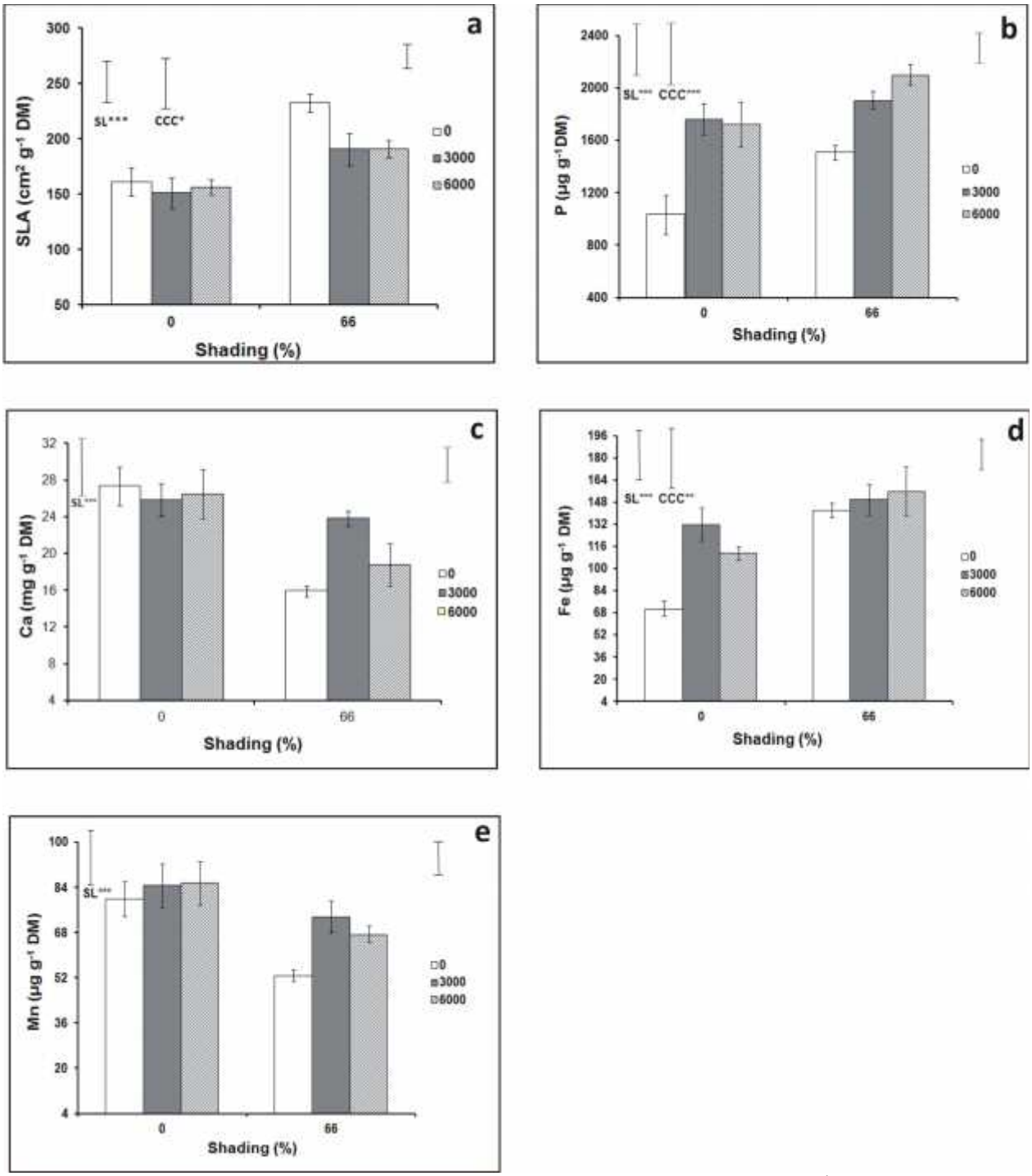

Figure 1. Effect of chlormequat chloride (CCC) concentration (0, 3000 and 6000 mg L^{-1}) on (a) specific leaf area (SLA) and leaf concentrations of (b) phosphorus (P), (c) calcium (Ca), (d) iron (Fe) and (e) manganium (Mn) on dry matter (DM) basis of *Lantana camara* L. subsp. *camara* plants at the shading levels (SLs) 0% and 66%. Bars on each column represent the standard error of the mean (n=6). The vertical bar at the right represents the standard error of the analysis. The vertical bars at the left represent the Least Significant Difference (LSD) values at $P = 0.05$ for the main effects of SL and CCC (*, **, *: significant at $P \leq 0.001$, ≤ 0.01 and ≤ 0.05, respectively). The absence of LSD bars indicates non significance.**

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