Environmental conditions and drenched-applied paclobutrazol effects on lantana specific leaf area and N, P, K, and Mg content

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Lantana camara L. is used extensively in many countries as an ornamental plant species but limited information is provided about the fertilization of this plant, especially after treatments with the triazole paclobutrazol (PBZ). The effects of drenched-applied PBZ at concentrations of 0, 40, and 80 mg L^{-1} on specific leaf area (SLA) and leaf N, P, K, and Mg concentrations of *L. camara* L. subsp. *camara* (lantana) plants were examined under different environmental conditions, resulted from different shading levels, 0% (daily light quantity of 27.8 mol m^{-2} d^{-1}) and 66% (9.4 mol m^{-2} d^{-1}) in glasshouse experiments in Attica, Greece. The concentration of leaf N was affected by the PBZ treatment ($P < 0.01$), while the shading level affected K and Mg concentration ($P < 0.01$). Plants treated with the same PBZ concentration presented higher SLA with 66% shading compared to 0% shading. The more the PBZ concentration, the lower and the higher were the SLA and leaf N respectively, at both shading levels. Leaf P, in general, increased with increasing PBZ concentration and shading as did K and Mg at 66% shading compared to 0% shading. At the end of the experiment all plants were healthy with no visible symptoms of nutritional deficiency. The findings of our study could be useful in establishing a fertilization program for *L. camara* plants treated with different drenched-applied PBZ concentrations under various environments.

Key words: *Lantana camara*, paclobutrazol, plant nutrition, shading, specific leaf area.

INTRODUCTION

Paclobutrazol (PBZ), chemically named (2*RS*, 3*RS*)-1-(4-chlorophenyl)-4,-4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl) pentan-3-ol (Davis et al., 1988), is a growth regulator of the triazole class which may influence plant metabolism by interfering with the three steps in ent-kaurene oxidation pathway, inhibiting thus the formation of ent-kaurenol, ent-kaurenal, and ent-kaurenoic acid. Finally, gibberellins (GAs) biosynthesis is inhibited (Graebe, 1987) and this fact leads mainly to plant growth retardation (Gianfagna, 1990; Jaleel et al., 2010). Therefore, PBZ exhibits retarding activity on a great variety of ornamental species e.g. marguerite (*Argyranthemum ×hybrida* 'Sunlight'), trailing petunia (*Calibrachoa × hybrida* 'Callie Dark Blue'), petunia (*Petunia × hybrida* 'Cascadias Vivid Red'), pale fanflower (*Scaevola albida* (Sm.) Druce 'Jacob's White') and garden verbena (*Verbena × hybrida* hort. ex Groenl. & Rümpler 'Rapunzel Red') (Blanchard and Runkle, 2007), and on many other plant species e.g.

wild cherry (*Prunus avium* L.) (Predieri et al., 2003) and potato (*Solanum tuberosum* L.) (Esmaielpour et al., 2011).

Lantana camara L. is a perennial widespread shrub exhibiting great ornamental potential with many uses in medicine (Zandi-Sohani et al., 2012). One subspecies of this plant, *L. camara* L. subsp. *camara* (lantana) is used widely for landscaping in several countries (Matsoukis et al., 2003). It has been reported that PBZ as a drench, especially at the concentration of 80 mg L^{-1} contributes to the production of attractive small potted lantanas with increased flowering potential (Matsoukis et al., 2001) but, to our knowledge, literature provides no information on the impact of drenched-applied paclobutrazol on other important parameters of lantana growth, such as specific leaf area (SLA) and mineral content (MC).

The projected leaf area per unit leaf DM (SLA) (Evans and Poorter, 2001), is an easily estimated parameter (Meziane and Shipley, 1999) and it is correlated with many other plant parameters e.g. relative growth rate (Garnier, 1992), photosynthetic capacity (Mendes and Marenco, 2010), and leaf palatability (Schädler et al., 2003). Therefore, SLA can be considered as an informative summary parameter on many aspects in plant metabolism. Also, the essential nutrients influence in a high degree the plant development by participating in many stages of plant metabolism (Moore et al., 1995) and their deficiencies alter physiological processes, reduce plant growth, often before visible symptoms appear, and cause morphological changes and injuries. Leaves are particularly sensitive

to nutrient deficiencies (Pallardy, 2008) and leaf tissue analysis is a well established method used to assess plant nutrient requirements (Van den Driessche, 1974; Vitousek et al., 2010). The knowledge of these requirements may be used for the establishment of appropriate plant fertilization programs. However, to our knowledge, no fertilization programs are available for lantana plants treated with PBZ or not. The need for proper fertilization of PBZ-treated lantanas is of great importance to create a highly quality flowering aesthetic plant and maintain it as much as possible. Limited information has been reported on N fertilization of *L. camara* L. 'New Gold' (Shurberg et al., 2012). For lantanas, the only information regarding triazoles and mineral nutrient content, to our knowledge, has been reported by Matsoukis et al. (2007; 2009).

Light availability can be changed by the use of shading materials above plant canopies, causing thus a change in environmental conditions which has been reported to having an effect on plant nutrition (Pequerel et al., 1997). The use of shading materials above plants in glasshouses in the summer period in countries characterized by high values of solar radiation e.g. Greece, is a usual practice for a lot of growers. By this way, they protect their cultivations from the direct effect of solar radiation and especially from the resulting quick increase of air temperature in the aforementioned period (Chronopoulou-Sereli and Flocas, 2010). The objectives of the present study focused on the effect of drenched-applied paclobutrazol, at concentrations of 0, 40, and 80 mg L^{-1}, on specific leaf area and on leaf N, P, K and Mg content, on a dry mass basis, of lantana plants under different shading levels (0% and 66%) in glasshouse experiments.

MATERIALS AND METHODS

Lantana plants derived from 16 to 18 cm mid-stem cuttings, were grown in a glasshouse in Attica (37°48'20" N, 23°57'48" E), Greece, as described by Matsoukis et al. (2001). Approximately 2 wk after their pinching at the second internode from the tips, when the new-developing stems had a length of about 1 cm (24 June), lantanas were treated with PBZ (250 g active ingredient [ai] per liter, Zeneca, Wilmington, Delaware, USA) solutions of 0 (control), 40 (4 mg ai per pot) and 80 (8 mg ai per pot) mg L^{-1} once as drenches at the rate of 100 mL per pot. No leaching phenomena were observed. At the same time, lantana plants were placed in two plots where different levels of photosynthetic flux density (PFD) were applied. Thus, there was a plot covered with black, dense woven net (66% shading level; model 201, Manioudaki Bros S.A., Athens, Greece). A non-shaded plot (0% shading level) was also included. Air temperature and relative humidity were monitored by two dataloggers with sensors for these parameters (Wilh. Lambrecht GmbH, Goettingen, Germany). PFD measurements were taken hourly during daytime, from 05:00 to 16:00 h, at the top of the plant

canopies, using two quantum sensors (LI-COR, Lincoln, Nebraska, USA). The two PFD 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 (RH) showed its higher value (70%) at 66% shading without differing more than 5.3% between shading levels. Plants irrigated as needed, with water containing K at 0.05 meq L^{-1}, Ca at 2.50 meq L^{-1}, Mg at 3.0 meq L^{-1}, HCO_3 at 5.0 meq L^{-1}, Na at 0.50 meq L^{-1} and 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, 5-mo after the beginning (23 November), 512 leaves, with no signs of senescence, from each PBZ treatment (consisted of eight 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. The leaf area (LA) of each leaf (excluding the petiole) was measured with an area meter (LI-COR, USA). Leaves were then oven-dried in paper bags at 65 °C for 60 h till constant dry weight (DW), giving a DM. SLA was calculated as LA divided by DM.

Sub-samples used for the determination of SLA were also used for the determination of N, P, K, and Mg. Dry leaf samples were ground (40 mesh sieve) to a fine powder and N concentration was determined by micro-Kjeldahl method. For the analysis of P, K, and Mg, 0.5 g ground dry material was placed in porcelain crucibles and ashed in a muffle furnace at 550 °C for 4 h. Ashes were subjected to wet digestion in concentrated HNO_3. Potassium and Mg were analyzed with atomic absorption spectrometry while P was determined colorimetrically (Gasparatos et al., 2011). All analyses were made twice and an average value was calculated for each element and plant-replicate in each PBZ treatment.

The experiments were 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 PBZ concentration (including controls). For the SLA data as well as for the mineral element data, means were calculated for each experimental plant and studied parameter and used for ANOVA. The two (out of eight) extreme means of each PBZ treatment were excluded from data before analysis. For the figure, standard errors (SEs) 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). Statistics was performed using SPSS version 8.0 for Windows and MS Excel 2007. Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Specific leaf area ($P < 0.01$) and leaf P ($P < 0.05$) were affected by both shading level and PBZ treatment (Table 1). The concentration of leaf N was affected by PBZ treatment, while shading level had a noticeable effect on K and Mg concentration ($P < 0.01$).

Lantana plants grown under 66% shading presented higher SLA values, compared to 0% shading, when examining the same concentration of PBZ (Figure 1a). These SLA increases were more pronounced in controls, over two times the respective increases in other PBZ treatments, and they were due to the greater increases of LA than DM. It can be assumed that these LA increases, reflect a lantana strategy to increase its competitive ability under 66% shading. A similar hypothesis was suggested by Devkota and Jha (2010) for *Centella asiatica* (L.) Urb. plants grown at similar shading (70%), as these plants presented higher SLA values compared to plants grown at full light conditions. Increased SLA values were reported for PBZ sprayed lantanas and *L. camara* plants grown at 66% shading (Matsoukis et al., 2007) and 37% sunlight (equivalent to 63% shading) (Carrión-Takuri et al., 2011), respectively.

In our experiment, it was impossible to isolate the effects of air temperature (T) and RH from those of PFD on lantana SLA. However, although it was reported that T (Atkin et al., 2006) and RH (Van de Sanden and Veen, 1992) may have an impact on SLA, it seems logical to claim that the impact of the aforementioned environmental parameters on lantana SLA was minor, taking into account that these, in average, differed only by 1.5 °C (T) and 5.3% (RH) between the examined light environments. The aforementioned hypothesis is also supported by the fact that daily differences of T and RH between 0% and 66% shading, were considerably lower than those of PFD for the examined time period (data not presented). Therefore, we could consider PFD as the predominant environmental factor in lantana SLA at our experimental conditions. Evans and Hughes (1961) reported that SLA is greatly influenced by shading, and subsequently by PFD, during the period of leaf expansion.

The more the PBZ concentration, the lower was the SLA value in the experimental plants at both shadings

(Figure 1a), with controls to differ ($P < 0.05$) from all other treatments in each light environment. The SLA decrease was more pronounced in lantanas treated with PBZ at the concentration of 80 mg L^{-1} compared to control, irrespective of shading, with the maximum decrease at 66% shading (more than 34%). The SLA decrease of the PBZ-treated plants was due to the greater lantana LA reduction than the leaf DM reduction, a known characteristic of PBZ as triazole (Davis et al., 1988).

The reduction in LA of lantana plants could be attributed, firstly, to a reduction in cell elongation (CEL), and, secondly, to a reduction in cell proliferation (CPR) resulted by reduced GA synthesis in response to PBZ treatment. A similar hypothesis was suggested by Ahmad Nazarudin et al. (2012) for PBZ treated *Syzygium myrtifolium* (Roxb.) Walp. plants with regard to CEL reduction, and by Tekalign and Hammes (2005) for PBZ treated potato plants with regard to CPR reduction. Decreased SLA values were reported for lantanas after spray applications with PBZ (50 and 100 mg L^{-1}) at the same, as in our experiment, light environments (Matsoukis et al., 2007). Also, decreased LA:leaf DW ratios (i.e. SLA) were reported for pecan (*Carya illinoinensis* [Wangenh.] K. Koch) plants (Wood, 1984) after drenched applications with higher and lower PBZ concentrations than those of our study.

Shading level had no effect ($P > 0.05$) on N concentration (Table 1, Figure 1b) while increased leaf P concentrations were found at 66% shading compared to 0% shading among experimental plants drenched with the same PBZ concentration (Figure 1c). The impact of shading on leaf P increase was greater for the control (almost 46% more leaf P at 66% shading compared to 0% shading), taking into account its greater differences between examined light environments, in relation to other PBZ treatments. The K concentration in lantana leaves was higher at the low light environment (66% shading) in relation to 0% shading, irrespective of PBZ concentration (Figure 1d), as did the leaf Mg (Figure 1e). When examining the same PBZ concentration (including control) between the studied light environments, 66% shading appeared to have a greater impact on leaf K of treated lantanas with PBZ at 40 mg L^{-1}, because in this concentration, K exhibited the higher increase (Figure 1d). As for the Mg, the impact of shading on it was greater in controls and was progressively diminishing with increasing PBZ concentrations as the Mg differences between 66% shading and 0% shading became smaller.

The possible temporary dryness of the substrate surface at 0% shading during the hot days just before irrigation, may, in part, have an impact on macronutrients uptake of control lantana plants. A similar hypothesis was suggested by Matsoukis et al. (2007) for the leaf N of lantanas grown under full light conditions (0% shading), as these plants presented lower concentrations of this nutrient in relation to plants grown under 66% shading. In addition, lower

Table 1. ANOVA for effects of shading level and concentration of paclobutrazol (PBZ) on specific leaf area (SLA) and leaf macronutrient content (N, P, K, Mg), on DM basis, of lantana plant.

		SLA	N	P	K	Mg
		cm^2 g^{-1}	mg g^{-1}	µg g^{-1}	mg g^{-1}	
	df	F				
SL	1	20.02***	2.33 ns	4.79*	18.80***	21.02***
CP	2	14.62***	23.01***	7.48**	0.68 ns	0.37 ns
SL × CP	2	3.13 ns	0.45 ns	0.963 ns	0.36 ns	0.07 ns

df: degrees of freedom; F: variance ratio; SL: shading level, 0% and 66%; CP: concentration of PBZ, 0, 40, and 80 mg L^{-1}.
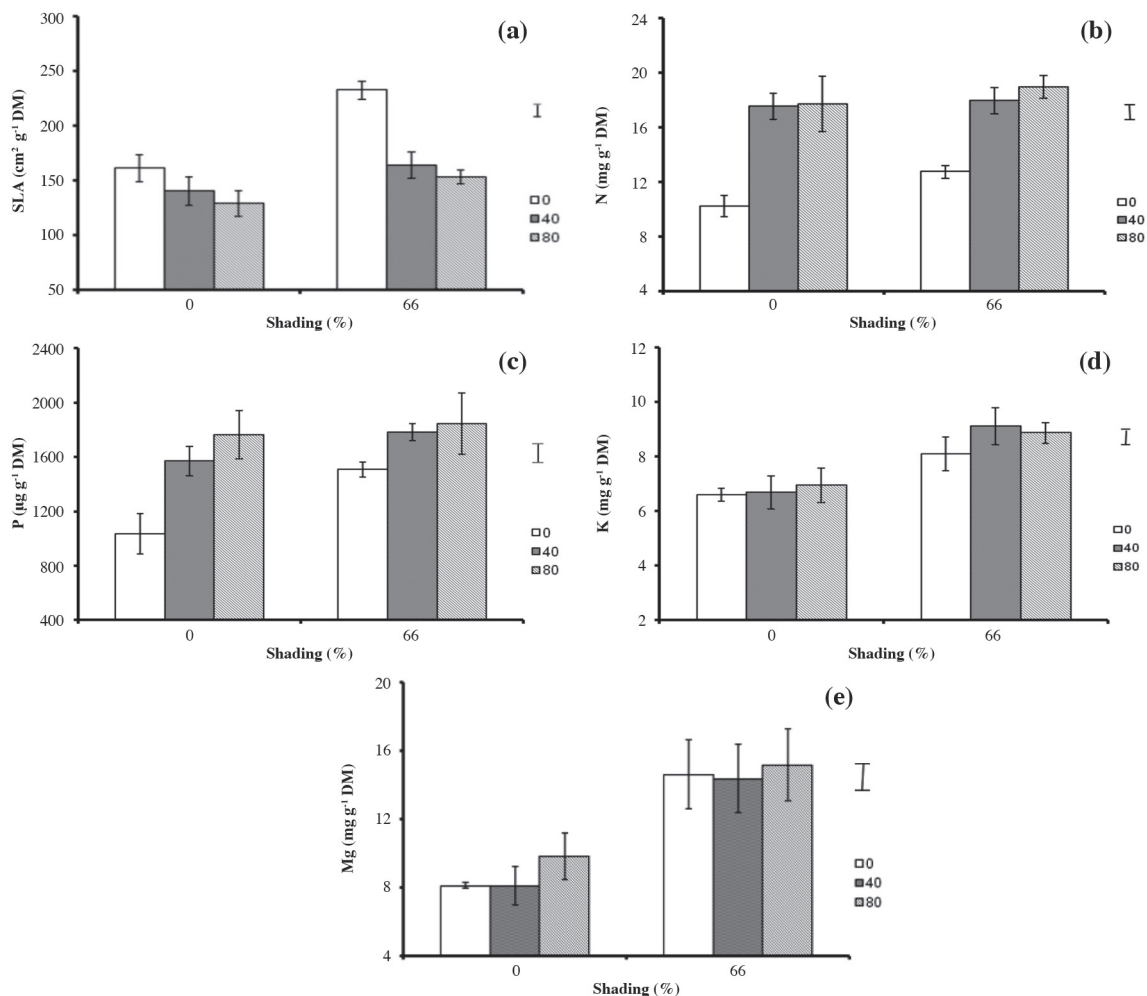***, **, *: significant at $P < 0.001$, < 0.01 and < 0.05, respectively; ns: non significant ($P > 0.05$).

Bars on each column represent the standard error of the mean (n = 6). The vertical bar at the right is the standard error of the analysis.

Figure 1. Effect of paclobutrazol concentration (0, 40, and 80 mg L⁻¹) on (a) specific leaf area (SLA) and leaf concentrations of (b) N, (c) P, (d) K, and (e) Mg on DM of lantana plants at the shading levels 0% and 66%.

P concentrations of the aerial part of yellow bluestem (*Dichanthium aristatum* [Poir.] C.E. Hubb.) were reported by Cruz (1997) under full sunlight compared to shade (30% up to 70%) during the driest regrowth cycle of this plant. Increased leaf P and leaf K concentrations have been reported in PBZ-sprayed lantanas (with 50 and 100 mg L⁻¹) grown at 66% shading compared to 0% shading (Matsoukis et al., 2009) while, to our knowledge, no comparable studies have been reported with other plant species with regard to the impact of PBZ and shading on leaf minerals.

Lantana controls exhibited the lowest leaf N concentration in relation to the other PBZ treatments at both shading levels (Figure 1b). The more the PBZ, the higher leaf N, in general, was found in lantana plants, as did the P concentration irrespective of shading (Figure 1c). The increased concentrations of leaf N and P of lantana with the increased PBZ concentrations in the examined light environments could be attributed to actual

changes in uptake and/or allocation of these elements inside the plant (Davis et al., 1988). Leaf K exhibited slight increases with the increased PBZ concentrations only at 0% shading, while this nutrient showed no pattern of change at 66% shading (Figure 1d), as did the leaf Mg in both light environments (Figure 1e). In a previous work (Matsoukis et al., 2007), increased leaf N concentrations of lantanas, in general, were reported with the increased PBZ concentrations (50 to 500 mg L⁻¹ as a spray) at 0% and 66% shading. In addition, increased leaf P values were reported on PBZ-sprayed lantana plants (with 50 and 100 mg L⁻¹) compared to control while the leaf K for the aforementioned plants showed no pattern of change, at the examined light environments (Matsoukis et al., 2009).

Literature provides no comparable studies as regards to other lantana species treated with PBZ, on concentration of Mg in their leaves. Higher concentrations of drench-applied PBZ than the examined ones have been reported to increase the concentration of leaf Mg (on a DM basis)

in peach (*Prunus persica* [L.] Batsch) trees (Pequerel et al., 1997) as well as to increase or not (Wieland and Wample, 1985) the leaf concentration of this nutrient in potted apple (*Malus domestica* Borkh. 'Topred Delicious') plants compared to controls (0 mg ai) under glasshouse conditions.

All experimental plants of our study, as confirmed by visual observations, were healthy with no symptoms of nutritional deficiency. To our knowledge, no foliar nutrient sufficiency ranges are available from literature on *L. camara* plants while PBZ may have an impact on fertilizer requirements in production plans (Davis et al., 1988) for lantanas. Our findings could contribute towards the creation of a fertilization program for this plant species which is very popular in Mediterranean countries as an outdoor plant.

CONCLUSIONS

In conclusion, as the shading increased from 0% to 66%, specific leaf area, leaf P, K, and Mg in lantana plants increased. The increased paclobutrazol concentration caused a decrease in specific leaf area and an increase, in general, in leaf N and P of lantana plants at both shading levels (0% and 66%). Our findings could contribute to the establishment of a fertilization program for *L. camara* plants in relation to different paclobutrazol concentrations and environmental conditions for the creation of high quality plants.

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