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Avoiding the use of plant growth regulator in geranium production by application of a cyclic deficit irrigation strategy

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Abstract

Plant growth regulators (PGRs) are commonly used in ornamental plant production to improve the decorative value of the plants and to meet marketable targets. The PGRs mostly used in ornamental plant culture are chemical growth retardants that control the size of plants, improve compactness and enhance flowering. However, the use of PGRs has been restricted under current legislation, and modified culture practices should be implemented to produce the desired quality of plants. Ornamental plant quality traits are determined by the genetic background of the plant and environmental conditions such as water availability. In the present study, the responses of growth and flower production in geranium (*Pelargonium peltatum* L.) subjected to cyclic deficit irrigation (CDI) were characterized to evaluate the technique as an alternative to the application of a plant growth regulator (daminozide). The leaf water potential of plants under CDI was lower than in control and PGR-treated plants. Moreover, the aerial dry mass, stem dry mass, leaf number, leaf blade area, specific leaf area and stem number of plants under CDI and PGR-treated plants were similar. However, the percentage of plants with at least one opened flower and the number of inflorescences per plant were increased by CDI. The marketable quality of the plants subjected to CDI was higher than that of the PGR-treated plants. Moreover, the water use efficiency of plants under CDI was 21% higher than that of PGR-treated plants, leading to a 10% reduction in the total water consumption during production.

Key words: Daminozide, *Pelargonium peltatum*, water stress.

Introduction

Chemical growth regulators are commonly used in potted plant production to control shoot elongation and to meet the quality criteria of the markets (Lodeta *et al*., 2010; Bañon *et al*., 2009; Krause *et al*., 2003). Daminozide (N-dimethylamino succinamic acid) is used on a variety of ornamentals to produce more compact plants. The compound is absorbed by the leaves and translocated throughout the plant (Moore, 1968). It acts by inhibiting synthesis of gibberellic acid (mainly GA_1 and GA_8) in the plant (Brown *et al*., 1997), thereby controlling excessive growth. The inhibitory effect of daminozide is greatest immediately upon application, and the effect becomes less pronounced thereafter, therefore continued growth regulation is accomplished by reapplication of the compound every 10 to 14 days (Rothenberger, 1964). Daminozide must therefore be applied more than once in order to produce a good level of growth retardation in most pot plants.

Although daminozide is relatively non-toxic to mammals (the oral LD_{50} in rats is 8400 mg kg^{-1}, the dermal LD_{50} in rabbits is > 1600 mg kg^{-1} and the inhalation LC_{50} in rabbits is > 147 mg L^{-1}; Meister, 1992), the principal health concern related to use of daminozide is the carcinogenic potential of unsymmetrical dimethyl hydrazine (UDMH), a contaminant and a metabolite of daminozide. UDMH is formed in the body during food processing, or when spray mixes containing daminozide are left standing in the mixing tank. Commercial daminozide contains 0.005% UDMH (US EPA, 1992). A metabolic study in swine showed that 1% of ingested daminozide is converted to UDMH. The US Environmental Protection Agency (1992) estimates that 0.012% of a daminozide solution converts to UDMH when allowed to stand in a tank for 24 hours. In female rats supplied with UDMH in their drinking water at concentrations of 0, 1, 50 or 100 ppm for 2 years, there was a significant dose-related increase in liver tumors (US EPA, 1992).

All use of daminozide on food crops was voluntarily cancelled by the manufacturer in 1989 and the product is currently registered only for use on ornamental and bedding plants. However, given the potential toxicity of the metabolite, alternative techniques for controlling plant growth are required. As plants grow, most of the increased size and weight is due to increased water content (Kramer and Boyer, 1995), and it is well known that water stress influences plant growth at various levels, ranging from cell to plant parts. When water is limiting, a number of plant functions are inhibited. Growth is one of the most water-sensitive physiological processes and leaf growth is one of the first types of growth to decrease, before photosynthesis is affected (Boyer, 1970).

The above-mentioned concept has been applied in agriculture for about twenty years, in the form of novel methods of irrigation scheduling such as keeping plants under a slight water deficit, the so-called ‘regulated deficit irrigation’ technique (Chalmers *et al*., 1986) or supplying irrigation alternately to different parts of the root system, leading to the ‘partial root-zone drying’ technique (Dry and Loveys, 1998). Two main techniques have been used to control plant growth in the production of greenhouse ornamentals: regulated deficit irrigation (Van Iersel *et al*., 2010; Álvarez *et al*., 2009; Blanusa *et al*., 2009) and cyclic deficit irrigation (Niu *et al*., 2007; Hansen and Petersen, 2004; Petersen and Hansen, 2003), in which, the water content of the growing medium alternates between container capacity and a very low soil water potential, sometimes leading to visible signs of wilting.

The objective of the present study was to characterize growth and flower production in geranium plants under cyclic deficit irrigation, which is a possible alternative to the application of plant growth regulators, and to evaluate any environmental advantages of the technique.

Materials and methods

Crop conditions: Individual rooted cuttings of *Pelargonium peltatum* L. were transplanted to 1 L (diameter, 13.0 cm; height, 11.4 cm) containers filled with a commercial peat substrate (Floragard, KTS2, long fibres) and grown in an automated polycarbonate-covered greenhouse located at The Basque Institute of Agricultural Research and Development (NEIKER, A.B., Biscay, Spain, latitude 43º 17' N, longitude 2º 52' W, altitude 77 m). The pots were placed on benches at a density of 15 plants m^{-2}. Plants were fertigated by sub-irrigation with a nutrient solution containing a commercial fertilizer ($3N-2P_2O_5-3K_2O-0.4MgO$, $6SO_3$, 0.002B, 0.004Cu, 0.01Fe, 0.01Mn, 0.0002Mo, 0.002Zn). The electrical conductivity (EC) of the nutrient solution was 0.5 mS cm^{-1}, and the pH was adjusted to 5.0 with phosphoric acid. The duration of the fertigation treatments and their frequency were controlled by tensiometers (Lapton Control de Riego, Bizkaia, Spain) inserted into the middle of one container per treatment and connected to an automatic pump control system. Each replicate had its own independent tank (50 L) containing nutrient solution and a pump controlled by the tensiometers. The air-heating was set to 10 and 12 ºC night/day, and vent opening temperatures were 12 and 15 ºC night/day, respectively. The climate data measured inside the greenhouse are shown in Fig. 1.

Treatments: Ten days after planting, the plants were subjected to one of three treatments: i) fertigation was started when the tensiometer reading reached a substrate water potential equal to -5 kPa (control); ii) plants were fertigated when the water potential of the substrate reached -5 kPa and were sprayed (1000 L ha^{-1}) three times (every 15 days) with a 0.5% solution of daminozide (N-dimethylamino succinamic acid) (PGR-treatment), or iii), plants were fertigated when the water potential of the substrate reached -15 kPa (cyclic deficit irrigation treatment). Daminozide (B-nine) was purchased in powdered form (85.0% ww^{-1} active ingredient) from Uniroyal Chemical Co., Middlebury, CT, USA. In all treatments, fertigation ceased when the substrate water potential reached saturation. The water content of the growing medium alternated between container capacity and the value of the substrate water potential at which fertigation started. A typical time course of substrate water potential is reported elsewhere (Riga *et al.*, 2003). Plants subjected to cyclic deficit irrigation did not show any visible signs of wilting. Each treatment was applied to 56 plants.

Water retention properties of the substrate: The moisture characteristics of the substrate were determined using a tension table apparatus (De Boodt and Verdonck, 1972; Jamison, 1958) for water potentials between 0 and -10 kPa. The substrate water content at -15 kPa was estimated using van Genuchten's model (1980), as follows:

$$\Psi s = \frac{\left[((\theta_s - \theta_r)/(\theta - \theta_r))^{1/m} - 1\right]^{1/n}}{\alpha}$$

where, Ψs is the substrate water potential, θ is the water content, θ_s and θ_r are the saturated and the residual water content respectively, α, *n*, and *m* are dimensionless empirical hydraulic parameters. θ_r was measured after drying the substrate at room temperature for a week (θ_r = 4%). The van Genuchten's model was fitted to observed data by minimizing the sum of quadratic differences between observed and calculated data by iterative Newton-Raphson optimization. The substrate water retention data and fitted curve are shown in Fig. 2. The estimated value of the substrate water content corresponding to a water potential of -15 kPa was 43.62%.

Measured crop parameters: In order to determine biomass production, the plants (from n = 6 to n = 18) were harvested randomly at 30, 50 and 75 days after treatment. Dry weights were measured after drying fresh biomass at 70 ºC to constant weight (48 h). Leaves were counted and leaf area was measured as follows: harvested leaves were immediately photocopied (Xerox, model XD332), and images were scanned to count the number of total black pixels with an image analyser. The relationship between the total number of black pixels and the corresponding area was calculated from a pre-established calibration curve. The specific leaf area (SLA) was calculated by dividing total leaf area (cm^2) by total leaf dry weight (g). Total water consumption was measured weekly by weighing the tanks containing the nutrient solution. Water use efficiency of the culture was measured as dry weight produced per unit mass of water loss by evapotranspiration. Marketable quality was evaluated by the number of open flowers

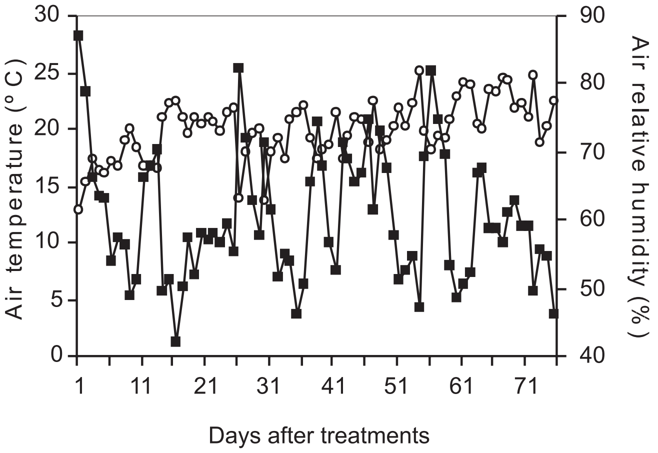

Fig. 1. Mean daily temperature (open circles) and relative humidity (closed squares) recorded inside the greenhouse.

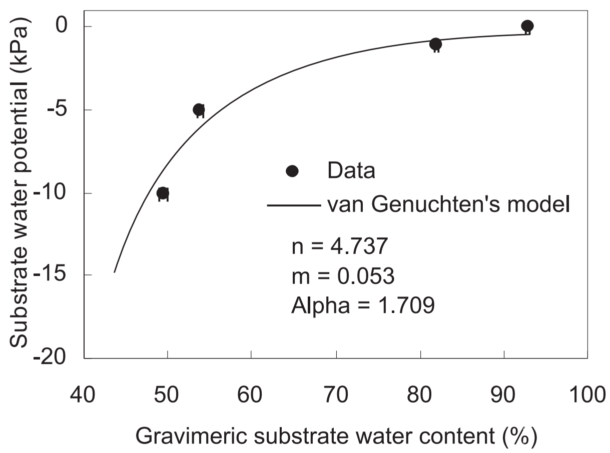

Fig. 2. Substrate water retention data obtained by the tension table technique, and curve fitted with Van Genuchten's model. *n*, *m* and alpha are empirical parameters. Means ± SD, (n = 3). When no bar is shown, it is included in the width of the symbol.

and by visual estimation of the global plant quality on the basis of local market requirements.

Leaf water status: Mid-day leaf water potential was measured between 1300 and 1500 (local time) using a pressure chamber (PMS Instrument Company, Oregon, USA) on the uppermost, fully expanded leaves of 5 plants per treatment (one leaf per plant). Cut leaves were immediately enclosed in plastic bags to prevent additional evaporation during handling of samples.

Statistical analysis: The data were analysed by the GLM and REG procedures in SAS (version 8.0. Differences between means were analysed by the Duncan's multiple range test.

Results

Leaf water potential: The mid-day leaf water potential was lower in plants under cyclic deficit irrigation (-15 kPa) than in control plants (-5 kPa) and PGR-treated plants (-5 kPa + PGR) (Fig. 3). Compared to control plants, the PGR treatment had no effect on Ψ_L.

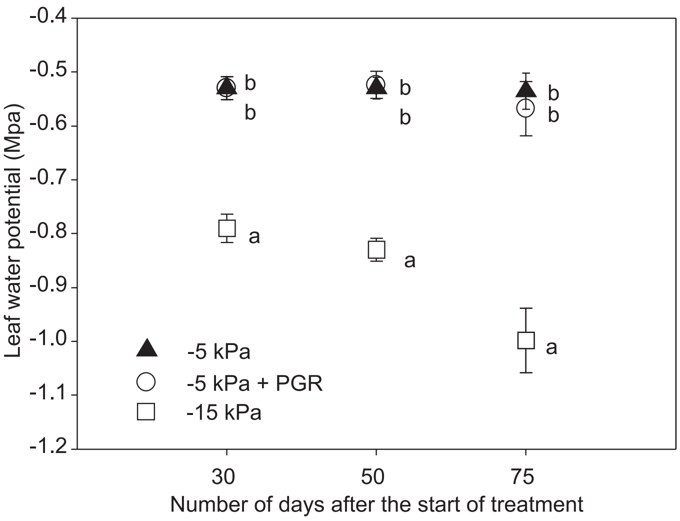

Fig. 3. Effect of treatments on midday leaf water potential measured between 1300 and 1500 (local time) on the uppermost, fully expanded leaves of 5 plants per treatment (one leaf per plant). Means ± SD. Means followed by the same letters are not significantly different at $P < 0.05$.

Plant growth parameters: Total aerial dry weights (leaves + stems) were affected by treatments after 75 days only (Fig. 4). The dry weight of control plants was significantly higher than that of the PGR-treated plants, confirming the positive effect of the daminozide treatment in reducing plant growth.

The treatments did not affect the leaf area per plant over the period of the experiment (Fig. 5). Nevertheless, 75 days after the start of treatments the plants under cyclic water deficit had more leaves than the control plants, but with no difference compared

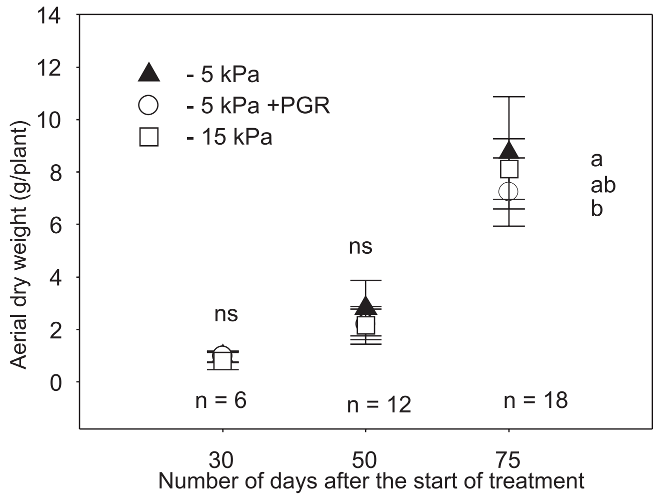

Fig. 4. Changes in total aerial dry weight over 75 days after the beginning of the treatments. Plants were irrigated when the tensiometer reading reached -5kPa (control) or -15kPa (water deficit). In addition, some plants irrigated at – 5kPa were sprayed every 15 days with a plant growth regulator (-5kPa + PGR). Means ± SD. Means followed by the same letters are not significantly different at $P < 0.05$. ns = not significant.

to PGR-treated plants (Table 1). For the same leaf area per plant, if the number of leaves increases depending on the treatment, then the leaf area per leaf decreases (Table 1). In addition, the SLA was lowest in plants under cyclic water deficit. There was a significant difference in SLA between the water deficit treatment and the control treatment.

Stem dry weights were only affected by treatments after 75 days (Fig. 6). The dry weight of control plants was significantly higher than that of PGR-treated plants. Stem dry weights represented about 10% of the value of total aerial dry mass over the period of the experiment (Fig. 4 and 6) and were not affected by treatments. However, 75 days after the start of treatment, the number of stems per plant was significantly higher in both cyclic water deficit and PGR-treated plants, than in control plants (Table 2). Nevertheless, there were no significant differences in the number of stems between the -15 kPa and PGR treatments. Moreover, the internode lengths were significantly lower in plants under cyclic

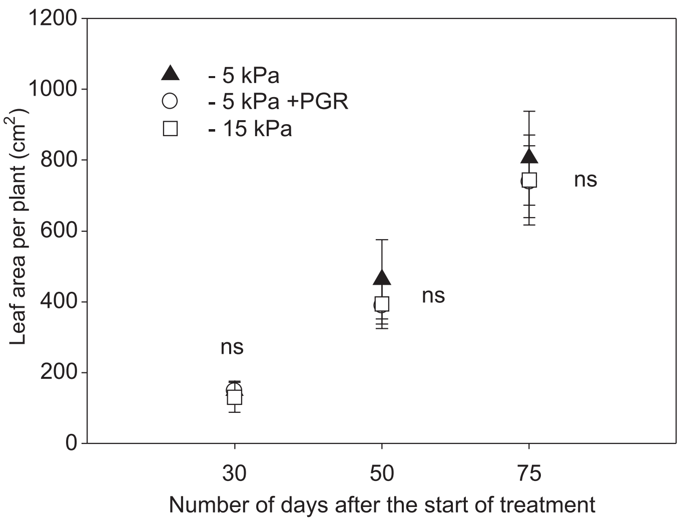

Fig. 5. Change in leaf area over 75 days after the beginning of the treatments. Plants were irrigated when the tensiometer reading reached -5kPa (control) or -15kPa (water deficit). In addition, some plants irrigated at – 5kPa were sprayed every 15 days with a plant growth regulator (-5kPa + PGR). Means ± SD. Means followed by the same letters are not significantly different at $P < 0.05$. ns = not significant.

Table 1. Effect of treatments on leaf number and leaf blade area at the end of the experiment (75 days)

Treatment	Leaves number	Leaf blade area (cm^2)	SLA (cm^2 g^{-1})
-5 kPa	30.00 (5.46) a	27.37 (2.10) a	3.77 (0.53) a
-5 kPa + PGR	31.36 (3.75) ab	23.35 (1.70) ab	3.15 (0.56) ab
-15 kPa	34.50 (4.62) b	21.96 (1.99) b	2.73 (0.60) b

SLA = specific leaf area. Values are means for 6 plants. Standard deviations are shown in bracket. Means followed by the same letters are not significantly different at $P < 0.05$.

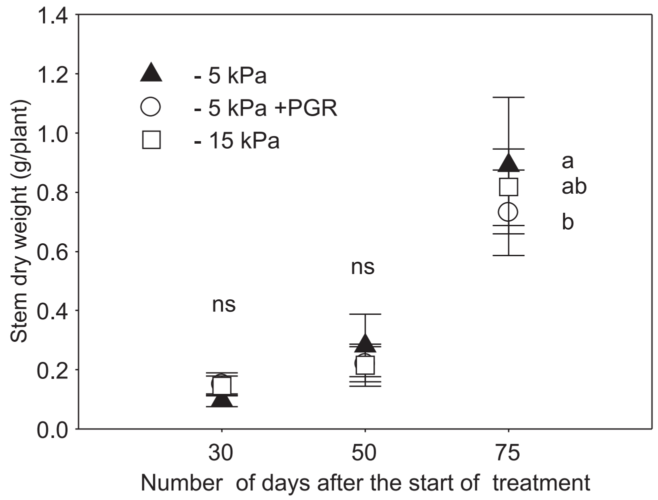

Fig. 6. Changes in stem dry weight per plant over 75 days after the beginning of the treatments. Plants were irrigated when the tensiometer reading reached -5kPa (control) or -15kPa (water deficit). In addition, some plants irrigated at – 5kPa were sprayed every 15 days with a plant growth regulator (-5kPa + PGR). Means ± SD. Means followed by the same letters are not significantly different at $P < 0.05$. ns = not significant.

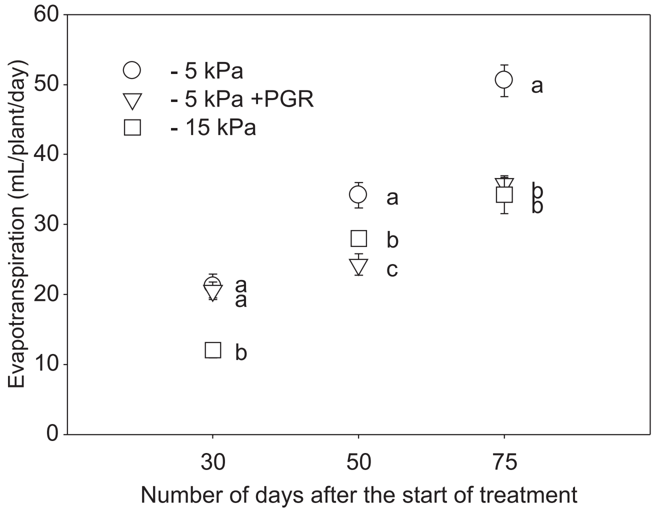

Fig. 7. Changes in daily evapotranspiration per plant over 75 days after the beginning of the treatments. Plants were irrigated when the tensiometer reading reached -5kPa (control) or -15kPa (water deficit). In addition, some plants irrigated at – 5kPa were sprayed every 15 days with a plant growth regulator (-5kPa + PGR). Means ± SD. Means followed by the same letters are not significantly different at $P < 0.05$. ns = not significant.

water deficit and treated with PGR than in control plants (Table 2). The internodal length was longest in control plants and shortest in PGR-treated plants.

Water consumption: Daily evapotranspiration of potted plants was affected early on by treatments (Fig. 7). At 30 days after the start of treatment, plants under cyclic water deficit showed the lowest evapotranspiration values, about two times lower than in control and PGR-treated plants. At this time, PGR treatment did not yet have any effect on evapotranspiration, in comparison with control plants. From day 50, evapotranspiration was lower in PGR-treated plants than in control plants and higher than in water stressed plants. After 75 days, significant differences were found only between control plants and the other two treatments. Thus, the highest value of total evapotranspiration over the whole period of the experiment was observed in control plants (Fig. 8a), whereas,

Table 2. Effect of treatments on stem number and on internode length at the end of the experiment (75 days)

Treatment	Stem number	Internodal length (cm)
-5 kPa	2.76 (0.63) a	3.76 (0.48) a
-5 kPa + PGR	3.21 (0.65) b	2.85 (0.46) b
-15 kPa	3.46 (0.68) b	3.22 (0.34) c

Values are means for 13 plants. Standard deviations are shown in brackets. Means followed by the same letters are not significantly different at $P < 0.05$.

Table 3. Effect of treatments on percentage of plants with at least one opened flower (OF) and on number of inflorescences per plant (IP) at the end of the experiment (75 days)

Treatment	OF (%)	Number of IP
-5 kPa	45.1 (5.2) a	2.12 (0.04) a
-5 kPa + PGR	44.9 (4.5) a	2.36 (0.17) ab
-15 kPa	62.9 (6.2) b	2.50 (0.09) b

Values are means for 13 plants. Standard deviations are shown in brackets. Means followed by the same letters are not significantly different at $P < 0.05$.

there was no difference between water stressed and PGR-treated plants. Total evaporation in the plants subjected to cyclic water deficit or PGR treatment was respectively 32 and 23% lower than in control plants.

Plant under water deficit showed significant higher WUE values (expressed as the ratio of production of aerial biomass against total evapotranspiration (Jones, 2004) than control and PGR-treated plants (Fig. 8b). The WUE values were 21 and 27% higher

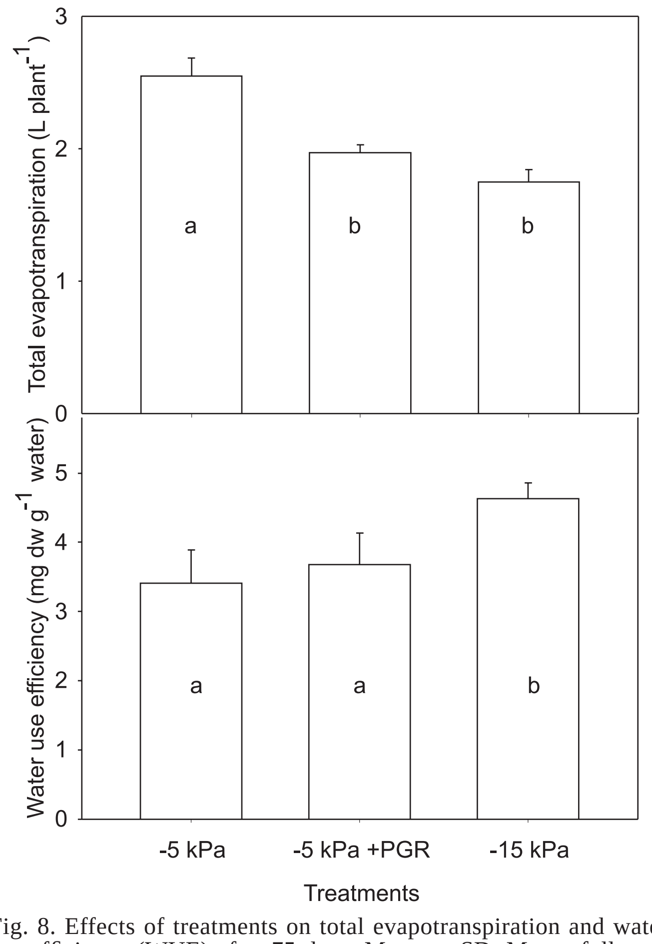

Fig. 8. Effects of treatments on total evapotranspiration and water use efficiency (WUE) after 75 days. Means ± SD. Means followed by the same letters are not significantly different at $P < 0.05$. ns = not significant.

in water stressed plants than in PGR-treated and control plants, respectively. Nevertheless, PGR treatment did not affect the WUE value compared to control plants.

Marketable quality: Under cyclic water deficit, the number of plants with at least one opened flower at 75 days after the start of treatment was higher than in control and PGR-treated plants (Table 3), and about 20% more of the water stressed plants had one opened flower. In addition, plants under the cyclic water deficit treatment had about 34% more inflorescences per plant than control plants, and the same number as PGR-treated plants. Treatment with PGR did not affect either the number of plants with at least one opened flower or the number of inflorescence per plant.

At 75 days after the start of treatment, visual evaluation of the plant quality was carried out on the basis of the local market requirements. Compact plants with at least one opened flower, with four or more ramified branches, and leaves of normal size and colour, were considered as marketable. A large number of inflorescences per plant also increased the marketable quality.

Plants grown under cyclic deficit irrigation and PGR-treated plants displayed higher overall quality than control plants, with no differences between the treatments (data not shown). The control plants were not of marketable quality because of their lack of compactness and large leaves.

Discussion

Effects of treatments on leaf water status and plant growth parameters: The midday leaf water potential (ΨL) ranged between -0.8 and -1.1 MPa in plants under cyclic deficit irrigation, which is consistent with a previous report of ΨL values between -0.7 and -0.8 MPa for *Pelargonium hortorum* plants under moderate or severe water stress, respectively (Sanchéz-Blanco *et al.*, 2009). Reducing the water supply to plants affects leaf water status, and leaf growth is one of the first parameters that is limited, before photosynthesis is affected (Boyer, 1970). Under the experimental conditions in the present study, the leaf blade area and SLA values were lowest, and the number of leaves were highest in the water stressed plants. The negative effect of water stress on leaf blade area has also been reported for *Hibiscus rosa-sinensis* (Hansen and Petersen, 2004), miniature roses (Williams *et al.*, 1999) and *P. hortorum* (Sanchéz-Blanco *et al.*, 2009). However, although the number of leaves was lower in water stressed *P. hortorum* than in control plants, the water stressed *P. peltatum* in the present study had more leaves because of a higher value of stem number than well watered plants. In addition, the SLA of water stressed leaves was lower because of a significant decrease in leaf blade area. Similar SLA response to water stress had been reported for legume species (Villagra and Cavagnaro, 2006). The SLA reflects aspects of leaf morphology, such as leaf density and thickness. Leaves tend to be more dense under water deficit than under well watered conditions, which leads to a decrease in SLA (Navas and Garnier, 2002; Castro-Díez *et al.*, 2000).

In plants treated with the plant growth regulator daminozide the leaf water potential was similar to that in control plants, the internode length was shorter and stem number was higher; treatment with daminozide did not affect the other leaf parameters measured. It is well known that gibberellins regulate growth (by increasing the rate of stem elongation), and as daminozide inhibits synthesis of these hormones (Brown *et al.*, 1997), the main effect of daminozide is to reduce the internode length, resulting in more compact plants.

Effects of treatments on water consumption: The daily and total evapotranspiration values were lowest, and the WUE values were highest in the *P. peltatum* plants under water deficit. The main effect of water deficit is to reduce the vegetative growth, leaf stomata conductance and transpiration, leading to an increase in the WUE as long as the water deficit is not too severe. When water is limited, the productivity of a plant that uses a finite water supply most efficiently would be positively affected (Loveys *et al.*, 2004). Plants produced with sufficient water supply had larger leaf areas and consequently these plants displayed the highest water consumptions and the lowest WUE. Interestingly, from 50 days after the start of the daminozide treatment, daily evapotranspiration in the treated plants was lower than in control plants, and after 75 days, the values decreased to the same level as in the water stressed plants, although the total leaf area was the same in the daminozide-treated plants and in the other plants. In addition, total evapotranspiration was lower in daminozide-treated plants than in control plants, but with no difference in the WUE values. Reports on the effect of daminozide on the water plant status are rather limited. However, in *chrysanthenum*, daminozide treatment was found to affect the stomata index, and the length and width of the stomata, resulting in leaves with xeromorphic properties (Kilic *et al.*, 2009).

Effects of treatments on marketable quality: The results of the present study showed that deficit irrigation produced the highest percentage of marketable plants and the highest percentage of inflorescences in *P. peltatum*, as previously reported (Riga *et al.*, 2003). In the case of *P. hortorum* a moderate water deficit (midday leaf water potential $\Psi_L \approx$ -0.7 MPa) did not affect the number of inflorescence or the number of open flowers per plant relative to control plants, while a severe water deficit ($\Psi_L \approx$ -0.8 MPa) decreased the values of these two parameters (Sanchéz-Blanco *et al.*, 2009). In the present study, the water stress conditions resulted in Ψ_L values between -0.8 and -1.1 MPa, suggesting that *P. peltatum* is more tolerant to water stress than *P. hortorum*. Many plant species can be induced to flower by the application of stress, *e.g.* water stress (Wada and Takeno, 2010). Water stress influences the number of flower buds and their opening depending on the plant species and the intensity of the stress.

In the present study, daminozide treatment did not affect the percentage of plant with at least one opened flower or the number of inflorescence per plant in comparison with control plants, as also found in a study on *Tagetes patula* (Krause *et al.*, 2003). In contrast, daminozide stimulated flowering in *Petunia hybrida*, *Impatiens walleriana* (Krause *et al.*, 2003) and in *Rhododendron sp*. (Meijón *et al.*, 2009). The mode of action of daminozide on flowering has not yet been elucidated.

Use of a cyclic deficit irrigation strategy proved to be a good alternative over the use of plant growth regulator for geranium (*P. peltatum*) production, as it maintained or even improved the marketable value of the plants. In comparison with the application of PGR, this technique reduced water consumption by 10%, and eliminated the cost of the PGR (about 3500 Euros per ha) and its application.

Without the need for daminozide and thus the absence of harmful residues, the working conditions of the growers would improve and risk of environmental contamination would decrease. The deficit irrigation strategy could therefore lead to an environmentally safer production of ornamental plants and to healthier working conditions.

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