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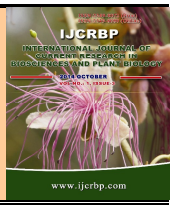


**Original Research Article**

# Effect of Nitrogen Fertilization and Amino Acid Foliar Sprays on the Quality and Yield of *Leucospermum cordifolium* cv. Tango

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**A b s t r a c t**

The protea cultivar Tango of the species *Leucospermum cordifolium* (L.) suffers a long pause in its growth produced by the stress of cutting the flowers. N fertilizers boost plants development and free amino acids applications have a preventing and recovering effect of plant stress. Taking into account these properties, 'Tango' plants have been treated with both kinds of substances in the field to verify their effect on the pause. The treatments consisted of 25 g of N per plant and 6 cL per L of Aminoquelant K (amino acids mixture) per year (Treatment 1), and 35 g of N per plant and 6 cL per L of Aminoquelant K per year (Treatment 2). The experiment followed a randomized block design with four replications per treatment. Potassium, Ca, Mg and Mn showed higher foliar concentrations in plants of Treatment 2. Foliar N content increased significantly in Summer and then decreased considerably, which implies that N applications should be made at the middle of the vegetative cycle. Treatment 2 resulted in statistically wider diameter and higher fresh weight of the flower buds in comparison with those of the Treatment 1. Removal of N and P, as well as chlorophyll content of lower leaves, increased with higher N fertilization and amino acid doses.

**K e y w o r d s**

Flower bud
Flowering stem
Foliar sprays
Nutrition
Protea

## Introduction

Proteas grow in poor soils and need low fertilization levels (Meynhardt, 1976; Thomas, 1982). However, their nutrient requirements differ so much among genera that the genus *Leucospermum* uptakes twice as many nutrient as the genus *Protea* (Montarone et al., 2003). Some researchers (Claasens, 1986, Parvin, 1986; Hernández et al., 2008) have even reported differences among species and cultivars of the same species. Some investigations have proved the beneficial effect of fertilizers on some species of

protea. Heinsohn and Pammenter (1986) found that high levels of $NH_4^+$, in combination with moderate concentrations of K and P, increased growth of *Leucadendron salignum*. Silber et al. (1998) reported higher total weight and growth of plants of *Leucadendron* 'Safari Sunset' after the application of N, P, K and micronutrients. With the same species Silber and Ben-Jaacov (2000) found higher foliar P contents and plant yields when the P concentration increased in the irrigation water.

In spite of the fact that the demand for fertilization by protea plants can be low, their nutrient output by the regular flower production is considerable (Fernández-Falcón et al, 2008; León et al, 2013). On the other hand, amino acids typically constitute 10 to 30% of plants dry matter, and they are used both for the production of new cell biomass and energy (Bush, 1993; Owen and Jones, 2001). Rhodes et al. (1999) reported the role of amino acids to overcome abiotic stress in plants, and their application to plants with this aim is a common practice in some crops (EcoGrower, 2013).

The plants of *Leucospermum cordifolium* cv. Tango suffer a long pause produced by the stress of cutting the flowers. Taking into account the above observations, the objective of this paper was to determine the effects of nitrogen fertilization and amino acids sprays on this pause and the improvement of the growth and production of the cultivar. Soil fertility and foliar nutrient behaviour along time were also studied. The nutrient removal of the crop in order to see their fertilization needs was determined, as well as foliar chlorophyll activity.

## Materials and methods

The study was carried out in 2011 with the cultivar 'Tango' of *Leucospermum cordifolium*, hybrid of *L. lineare* cv `Diadem´ × *L. glabrum* cv `Helderfontein´ (Littlejohn, 1995), in a commercial plantation located at the North East of La Palma (Canary Islands). The soil was an Inceptisol Andept. This zone has a temperate and humid climate, with mean temperatures between 12 and 20°C, and 500 to 800 mm/year of raining.

### Experimental design

The experiment followed a randomized block design with four replications per treatment, two lines of 30 plants each per replication.

The treatments consisted of two doses of ammonium sulphate (25 and 35 g/ plant/year) (Table 1) mixed with two doses of foliar sprayed amino acids (Table 2) with the following schedules:

In addition, according to the recommendations of Hernández et al. (2014), all the plants received (per $m^2$ and year): K: 16 g, Ca: 2.4 g, Mg: 8 g.

**Table 1. Nitrogen fertilization per treatment (g plant$^{-1}$)**

| Ammonium sulfate | Apr | May | Jun | Jul | Total per year |
|---|---|---|---|---|---|
| Treatment 1 (T1) | 5 | 5 | 10 | 5 | 25 |
| Treatment 2 (T2) | | 10 | 10 | 15 | 35 |

**Table 2. Amino acid application per treatment (cL L$^{-1}$)**

| Aminoquelant K | Apr | May | Jun | Jul | Nov | Dec | Total per year |
|---|---|---|---|---|---|---|---|
| Treatment 1 (T1) | | | | | 3 | 3 | 6 |
| Treatment 2 (T2) | 3 | 3 | | | 3 | 3 | 12 |

Plants were drip irrigated. Throughout the period May to July each plant received 8 to 10 L of water per week, while along the period of highest consumption (August) 20 L per plant and week were given. Irrigation water had an electrical conductivity of 0.31 dS/m and a pH of 8.3.

### Soil and plant samplings and analysis

*Soils:* Coinciding with the start and end of the vegetative stage (March 2011 and February 2012), soil samples were collected at a depth of 0 to 25 cm with an Eijkelkamp soil sampler. One composite sample per replication was taken (Porta et al., 1999). The samples were ground through a 2 mm mesh after air drying. A mixture of soil and water with a ratio of 2:5 was shaken and then allowed to settle for 10 minutes, after which it was used to measure the pH.

The Walkley and Black method to determined organic matter was followed, with the modifications made by the Comisión de Métodos Analíticos del Instituto de Edafología y Agrobiología "José M. Albareda" (1973). Soils were shaken within an ammonium acetate 1 M solution at pH 7, and then

available cations were determined in the extract by ICP PerkinElmer. Available P was determined by the Watanabe and Olsen (1965) method after extracting it by the Olsen et al (1954) method.

Texture was determined by the Bouyoucos method (López, 1987). Conductivity (EC) was measured in the saturated soil extract (López, 1987).

*Plants:* Four foliar samplings, one every two months, were carried out along the vegetative cycle, starting from the time when the shoots reached 16 cm after the spring pruning (usually in May), and ending in November (beginning of the harvesting season). The sampled leaves were the fully developed last emerged ones ($4^{th}$ or $5^{th}$ leaf counting from the apex). Four replications per treatment were sampled, each one consisting of leaves from 15 plants.

To study the nutrient distribution in the different organs of the flowering stem and the nutrient output throughout the crop, flowering stems of commercial quality were taken from each of the treatments in January 2012. Samples consisted of four replications with three flowering stems each. Lengths of these flowering stems were measured, and then they were cut into different parts: flower bud, leaves, and stem. Fresh weight and dry weight of each of these parts were determined.

The plant samples were washed in distilled water and dried in an oven at 80ºC, after which they were ground to powder. One g of the powder was ashed in an oven at 480ºC and then mineralized by dry ashing with 6 M hydrochloric acid (Chapman and Pratt, 1961). The levels of P, K, Ca, Mg, Cu, Fe, Mn and Zn were determined by ICP Perkin-Elmer. Nitrogen was determined by the Kjeldahl method (Cottenie, 1980).

### Growth measurements

*Stem elongation:* Stem growth (elongation) was measured weekly throughout eleven consecutive weeks in five stems from five plants per replication, starting from its base of inclusion in the carrier up to the apex of the bud.

*Flower buds growth:* After the flower buds reached a diameter of 2.5 cm (at the beginning of November), the growth of five flower buds per replication were determined weekly, by measuring with a gauge their lengths from the base to the apex until they ceased to grow longitudinally (January 2012).

*Flower production:* To determine the flower production, the stems (including the flower) were considered to be of commercial quality when they reached 30 cm or more in length, straight and without defects.

### Chlorophyll concentration measurements

Five plants per replication were selected at random to measure the chlorophyll concentration with a Minolta SPAD-502. Measurements were taken at midday from leaves growing at two levels (level 1 = $4^{th}$ to $5^{th}$ leaves; level 2 = $9^{th}$ to $10^{th}$ leaves) of the flowering stem. The leaves of level 1 coincided with those used for foliar samplings in September and November.

### Nutrient removal

The nutrient removal by the harvest of flowers was determined taking into account the production per square meter (an average of 25 flowering stems per $m^2$).

### Statistical analysis

Data were subjected to one-way variance analysis, using Tukey b tests at $p = 0.05$, Student's t test, Mann-Whitney U test, and correlations by SPSS 15.0 statistical software.

## Results and discussion

### Soils

The soils presented a sandy clay texture (Table 3), that is considered suitable for protea culture (Montarone, 2001; Hernández, 2008). The chemical analysis revealed acid soils, that proteas tolerate well (Cecil et al., 1995; Maier et al., 1995; Montarone, 2001), with the pH decreasing slightly with time, probably due to the fertilization with sulfates.

Organic matter contents were in general high (Álvarez et al., 2012), though the treatment with the higher nitrogen doses (T2) significantly decreased

it, perhaps due to an increase of organic matter decomposing bacteria. However, total N of the soil did not show appreciable differences between both treatments.

**Table 3. Physical and chemical characteristics of the soils**

| Treatment | Sampling | pH | mg kg$^{-1}$ $P_2O_5$ | g kg$^{-1}$ | | Available cations cmol kg$^{-1}$ | | | | E.C. dS m$^{-1}$ | Texture |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | O.M. | N | Ca | Mg | Na | | | |
| | 1st | 5.8 | 9 | 80 | 3.8 | 3.8 | 3.2 | 0.57 | 2.8 | 0.48 | Sandy clay |
| T1 | 2nd | 5.3 | 6* | 50* | 4.1 | 3.7 | 2.9* | 0.55 | 3.0* | 0.39 | |
| T2 | 2nd | 5.3 | 5 | 60 | 3.5 | 2.2 | 1.9 | 0.41 | 1.5 | 0.37 | |

*Denotes a significant difference at 5% level between treatments in the second sampling.

**Table 4. Foliar analysis of the plants from the different treatments and samplings.**

| Sampling | Treatment | g kg$^{-1}$ | | | | | | mg kg$^{-1}$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Mg | Na | Fe | Mn | Cu | Zn |
| May | T1 | 11.3 | 1.1 | 3.8 | 4.5 | 2.0 | 5.3 | 126 | 514 | 7 | 48 |
| | T2 | 12.0 | 1.1 | 3.7 | 5.0 | 2.1 | 4.8 | 142 | 627 | 7 | 54 |
| July | T1 | 13.8 | 0.7 | 4.4 | 4.9 | 2.3 | 6.6 | 47 | 468 | 5* | 39 |
| | T2 | 16.9 | 0.7 | 4.5 | 4.6 | 2.3 | 6.3 | 56 | 447 | 4 | 41 |
| Sept. | T1 | 9.5 | 0.8 | 4.4* | 5.3* | 2.9 | 6.1 | 46 | 755* | 4* | 49 |
| | T2 | 8.4 | 0.9 | 5.6 | 7.1 | 3.2 | 7.0 | 27 | 1054 | 2 | 55 |
| Nov. | T1 | 9.2 | 0.7 | 3.4 | 11.2* | 2.5* | 5.5 | 54 | 993* | 2* | 23 |
| | T2 | 8.8 | 0.6 | 2.9 | 19.7 | 2.9 | 4.8 | 56 | 1710 | 1 | 26 |

*Denotes significant differences at 0.05 level between data of the different treatments of the same sampling.

The concentrations of available phosphorus were low for cultures in general, but adequate for proteas (Thomas, 1980; Jamienson, 1985; Heinsohn and Pammenter, 1986; Cecil et al., 1995) especially for the studied cultivar 'Tango' (Álvarez et al., 2012).

Calcium and Mg presented low values that are suitable for proteas, while available K showed high concentrations. Available K and Mg were significantly lower in Treatment 1, and Ca presented a similar pattern. The salinity index ranged within suitable lower and higher points (Rodríguez-Pérez et al., 2001) in both treatments, and in the two samplings, with lower levels below the rank (1.7 dS m$^{-1}$) that these authors pointed out as critic for *Leucospermum cordifolium*.

## Leaves

Both treatments showed similar foliar N, P, Na, Fe and Zn concentrations in the four bimonthly foliar samplings carried out throughout the growing cycle (Table 4). Nitrogen increased significantly in July, decreased considerably in September and remained stable at the beginning of the flowering season (November) (Fig. 1). This implies to draw more attention to N applications at the middle of the vegetative cycle in order to meet the needs of the plants. With the exception of the July sampling, N stood in the optimum range for this cultivar (Álvarez et al., 2012). This is an interesting new contribution due to the scarce bibliographic information that exists in this respect.

The variation of P with time (Fig. 1) showed that the levels of this nutrient in both treatments were notoriously higher at the beginning of the growth of the plants (Spring), after which they fall along the growing cycle. The greater P needs of the plant at the beginning of the development (especially in the formation of roots) and at the time of formation of the flower buds (Mengel and Kirkby, 2001) in late summer explain this behavior.

Foliar K levels, in general, are higher than those reported by Alvarez et al. (2012) for this cultivar. In September its concentration in treatment 1 was significantly greater than that of the treatment with nitrogen and amino acids. K levels increased with time, especially in Treatment 2, but fell deeply in November (Fig. 1). This behavior agrees with that pointed out by Cecil et al. (1995) in *Leucadendron*, as well as Maier et al. (1995) in genus *Protea*, and Hernández (2008) in *Leucospermum*. It follows that most suitable time to fertilize with K is in Spring and Summer.

**Fig. 1. Foliar N, P and K levels throughout the study period (Different letters denote significant differences among samplings of the same variable at *p*=0.05).**

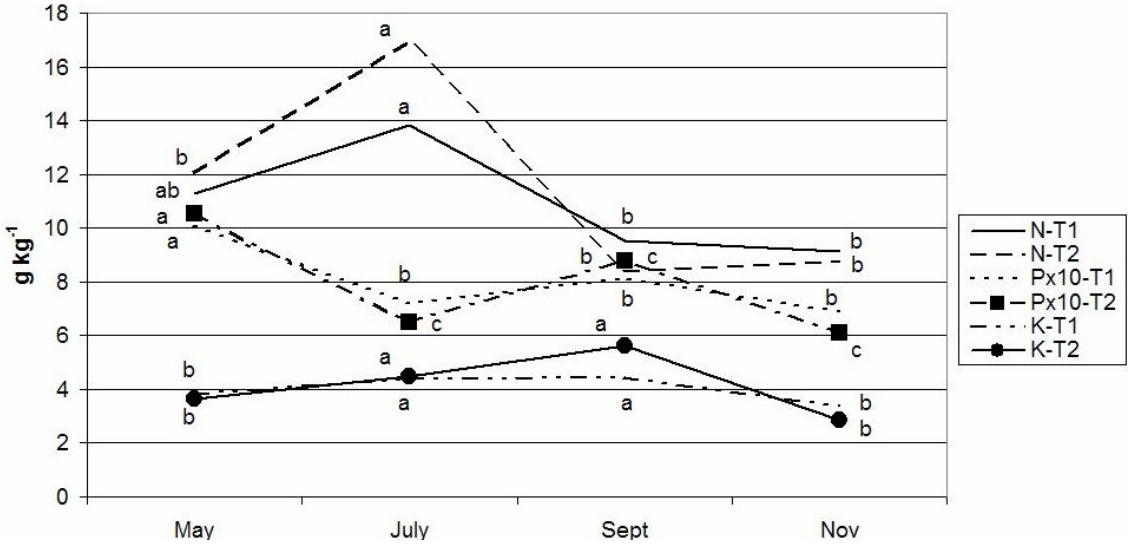
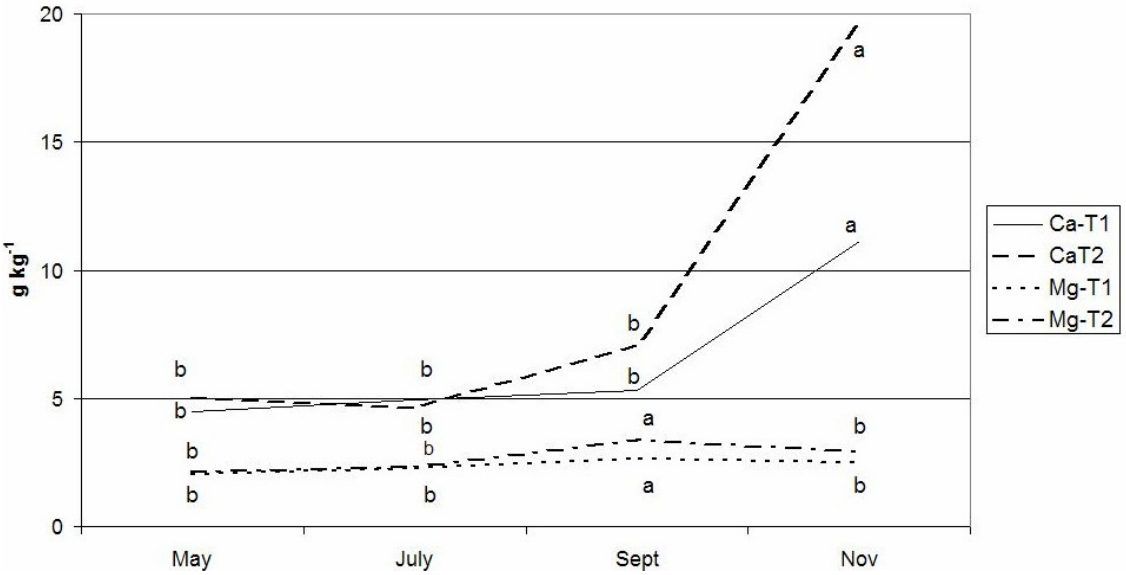



**Fig. 2. Foliar Ca and Mg levels throughout the study period (Different letters denote significant differences among samplings of the same variable at *p*=0.05).**




Plants generally require more Ca at the end of the vegetative cycle (Mengel and Kirkby, 2001), and this happens also with the studied protea cultivar (Fig. 2), where foliar Ca also presented higher concentrations in the treatment with nitrogen (Table 4). At this time foliar Ca also exceeded the standard levels reported by Alvarez et al. (2012), and the treatment with nitrogen presented significantly higher concentrations of this nutrient.

Maier et al. (1995), in genus *Protea*, and Hernández et al. (2008), in various cultivars of *L. cordifolium*, also found this pattern in foliar Ca.

Foliar Mg also increased in the treatment with nitrogen in the third sampling (Fig. 2). The values of Mg in May and July were within the range pointed out by Alvarez et al (2012) for this cultivar, but in September they exceeded it.

In the last two months of sampling there was a significant decrease in the ratio N/Ca in Treatment 2 with respect to the Treatment 1 (Table 5), which coincide with the lowest values displayed by each treatment over time. A similar behavior with respect to the variation with time was observed in the ratio K/Ca.

**Table 5. N/K, N/Ca, K/Ca ratios of foliar nutrients of the different treatments and samplings.**

| Sampling | Treatment | N/K | N/Ca | K/Ca |
|---|---|---|---|---|
| May | T1 | 2.97 | 2.53 | 0.86 |
| | T2 | 3.35 | 2.53 | 0.75 |
| July | T1 | 3.15 | 2.83 | 0.90 |
| | T2 | 3.78 | 3.79 | 0.99 |
| September | T1 | 2.19* | 1.85* | 0.84 |
| | T2 | 1.49 | 1.19 | 0.80 |
| November | T1 | 2.71 | 0.88* | 0.32* |
| | T2 | 3.32 | 0.50 | 0.18 |

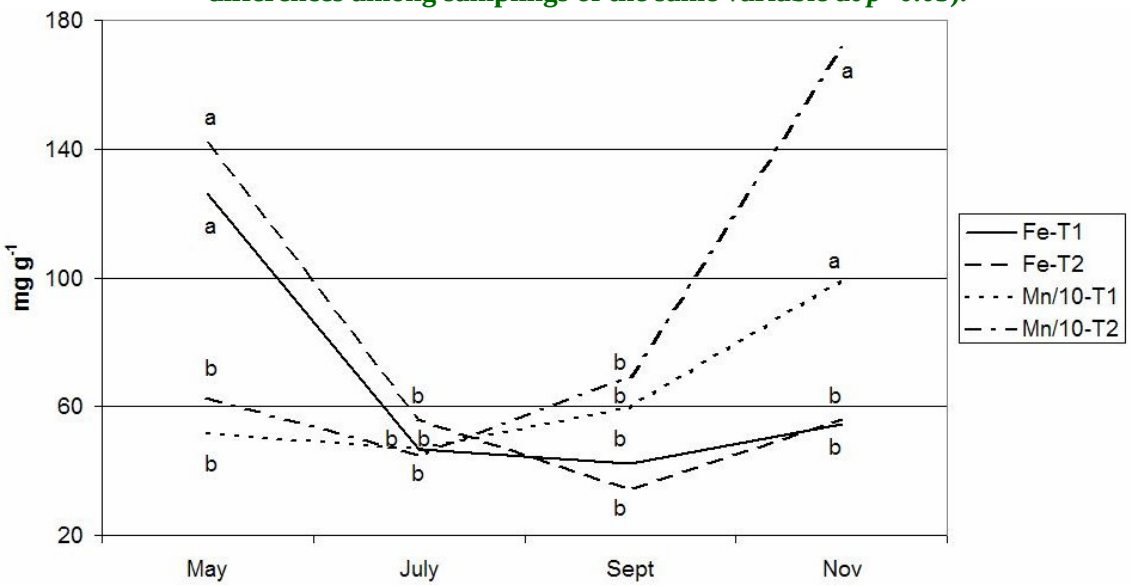
*Denotes significant differences at 0.05 level between data of the different treatments of the same sampling.

**Table 6. Flower bud measurements throughout the bloom season.**

| Treat-ment | Diameter of the flower buds (mm week$^{-1}$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1ª | 2ª | 3ª | 4ª | 5ª | 6ª | 7ª | 8ª | 9ª | 10ª | 11ª |
| T1 | 26.78 | 32.18 | 37.00* | 43.57 | 49.02* | 55.95* | 63.59* | 69.15* | 72.80* | 73.34* | 70.89* |
| T2 | 27.51 | 32.89 | 38.69 | 45.17 | 51.10 | 58.65 | 66.84 | 72.71 | 77.35 | 77.81 | 77.86 |

*Denotes significant differences at 0.05 level between data of the different treatments of the same sampling.

**Fig. 3. Foliar Fe and Mn levels throughout the study period (Different letters denote significant differences among samplings of the same variable at $p$=0.05).**

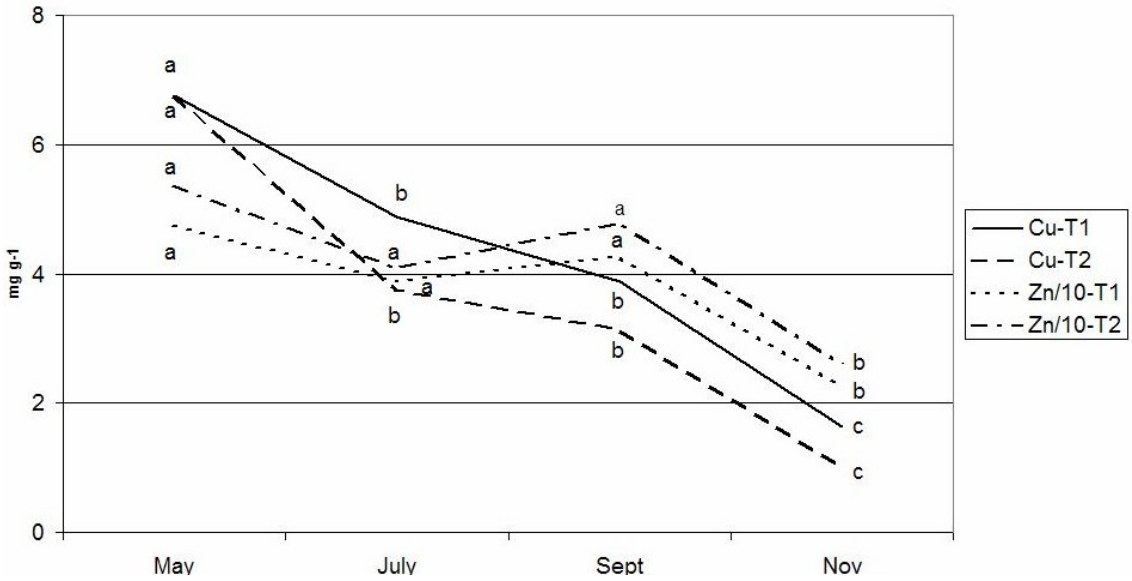



Sodium uptake was particularly high (Table 4), because its concentrations exceeded those of the other nutrients except Ca. Rodríguez Pérez et al. (2000 and 2001), in genera *Leucospermum* and *Protea*, and Hernández et al. (2008), in various cultivars of *Leucospermum cordifolium*, had reported the great capacity of absorption of Na that these plants have.

As far as micronutrients is concerned, Fe levels (Table 4) in the samplings of May and July exceeded the range reported by Alvarez et al. (2012), but in September and November samplings they fitted that range. At the beginning of the vegetative development (Spring) there was a great demand of Fe that decreased in Summer and in the Fall (Fig. 3), which suggests that Fe applications could be considered after pruning.

On the other hand, Mn (Fig. 3) showed a behavior opposite to that of Fe, since the needs of the plant remained stable from the beginning of the vegetative cycle till the end of Summer, and then increased towards the end of the stage of development of the plant. The Mn standard levels suggested by Alvarez et al. (2012) were lower than those found here. It should be noted that, in the last two sampling, Mn concentrations in the leaves of the plants treated with N were higher than those found in non-treated plants.

**Fig. 4. Foliar Cu and Zn levels throughout the study period (Different letters denote significant differences among samplings of the same variable at *p*=0.05).**




Copper presented notable differences between treatments in favor of the T1 (Table 4), in three of the samplings, showing a steady decrease in each treatment, from spring until the end of the vegetative cycle (Fig. 4). This trend means that Cu applications should be made in Spring. All the Cu values fit the range reported by Alvarez et al. (2012).

The highest concentrations of Zn appeared in May and July samplings, and then they significantly declined (Fig. 4), so that suitable seasons to fertilize with Zn could be Spring and Summer. Foliar Zn concentrations in the samplings of May, July and September exceeded those found by Alvarez et al. (2012).

## Growth parameters

*Diameter of the flower buds:* The plants that received the greatest nitrogen fertilization presented generally a statistically higher diameter of their flower buds in comparison with those of the Treatment 1 (Table 6). The difference reached a 8.9% at the end of their growth. Since nitrogen fertilization in proteas is being questioned, these results are of considerable interest in connection with a better quality of the flower bud.

*Elongation of the flowering stems:* This parameter determination was carried out throughout twelve consecutive weeks. Unlike what happened with the diameter of flower buds, the elongation of the stems did not show differences between treatments (Table 7). The reached final lengths fitted those of the current commercial standards of this cultivar.

*Fresh and dry weight of the flowering stems and flower yield:* Lengths and dry weights of the flowering stems did not present significant differences, not either the flower production (Table 8). On the contrary, the fresh weights of the flowering stems of the Treatment 2 were statistically higher than those of the Treatment 1, what could be related to the greater elongation of the flower buds. This results in a better quality of the flowers from the commercial standpoint.

**Table 7. Flowering stems elongation throughout the bloom season.**

| Treatment | Stem elongation (cm week$^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1ª | 2ª | 3ª | 4ª | 5ª | 6ª | 7ª | 8ª | 9ª | 10ª | 11ª | 12ª |
| T1 | 32.6 | 34.7 | 36.3 | 38.9 | 41.4 | 45.1 | 48.4 | 50.8 | 53.6 | 56.2 | 57.9 | 58.8 |
| T2 | 30.6 | 32.8 | 34.2 | 36.7 | 39.1 | 43.0 | 46.2 | 48.1 | 51.1 | 53.8 | 56.0 | 56.8 |

*Denotes significant differences at 0.05 level between data of the different treatments of the same sampling.

**Table 8. Length, fresh and dry weight, and number of flowering stems per plant.**

| Treatments | Length (cm) | Fresh weight (g) | Dry weight (g) | Number of flowering stems |
|---|---|---|---|---|
| T1 | 65.5 | 113.6* | 23.00 | 29.4 |
| T2 | 67.4 | 129.0 | 23.42 | 27.8 |

*Denotes significant differences at 0.05 level between data of the different treatments of the same sampling.

**Table 9. Nutrient removal by the harvested flowering stems.**

| Treatment | g $m^{-2}$ | | | | | mg $m^{-2}$ | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | Fe | Mn | Cu | Zn |
| T1 | 7.8b | 0.8b | 6.1 | 10.2 | 2.9 | 39.5 | 638 | 5.7 | 23.7 |
| T2 | 10.8 a | 1.2a | 8.0 | 11.8 | 3.4 | 44.1 | 974 | 4.7 | 19.5 |

The data in the columns followed by different letters are statistically different at the level of $p = 0.05$.

### Nutrient removal

In the cases of N, P, Ca, Mg, Fe and Mn (Table 9) a general trend higher removals by the plants that received the higher doses of N and aminoacids, though they were significant only for N and P.

### SPAD measurements

No clear differences of SPAD values (Table 10) between treatments appeared in the highest leaves (level 1), but significant differences were appreciated in the lowest leaves (level 2). This can be attributed to the fact that, in both samples, the chlorophyll activity was higher in plots with higher nitrogen fertilization, in accordance with what Chapman and Barreto (1997) and Loh et al. (2002) had observed in other cultures. The leaves of level 2 that belonged to Treatment 1 showed also a lighter green color than those of Treatment 2, which implies that the stems of this treatment showed higher commercial quality.

**Table 10. SPAD measurements.**

| Samplings | Treatments | Level 1 | Level 2 |
|---|---|---|---|
| 3 | T1 | 29.02 | 44.5 |
| | T2 | 29.25 | 50.8* |
| 4 | T1 | 48.83 | 65.6 |
| | T2 | 45.36 | 69.1* |

* Denotes significant differences at 0.05 level between data of the different treatments of the same sampling.

## Conclusions

Traditional Foliar nitrogen increased significantly in Summer, decreased considerably in September and remained stable at the beginning of the flowering season (November) (Fig. 1). This implies to draw more attention to N applications at the middle of the vegetative cycle in order to meet the needs of the plants. Given the limited bibliographic information in this area for *Leucospermum*, the contribution made in this regard is very important.

The needs of the plant in P were higher both in the time of the beginning of the development of the plant (particularly in the formation of roots) as in the time of formation of the flower buds (late summer. Significant higher flower buds size and fresh weight of the flowering stems of plants that received the Treatment 2 were detected. Chlorophyll activity as also higher in plants of Treatment 2, with the older leaves presenting darker green colour. These facts result in a better quality of the flowers from the commercial standpoint.

As nitrogen fertilization in proteas is being questioned, these findings are of considerable interest, because they are the only ones in the literature concerning the contribution of nitrogen and amino acids with a view to obtaining better productions. Nitrogen, P, Ca, Mg, Fe and Mn removal by the harvested flowers showed a trend to be higher in Treatment 2, though they were significant only in the cases of N and P.

The treatment with greater contribution of nitrogen and amino acids resulted in increased nutrient removal, indicating that a non-aggressive nitrogen fertilization favors a major widespread absorption of nutrients by the plants.

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