Original article

Use of multivariate statistics to determine fertilization practices that affect fruit quality of naranjilla (*Solanum quitoense* Lam.)

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Summary

Introduction – **Naranjilla (*Solanum quitoense* Lam.) is a native fruit of the Andes and is cultivated in several provinces of the Ecuadorian Amazon. It constitutes a crop of economic importance for the small farmers of this region of Ecuador. There is a lack of information on the nutrients required by this crop species in Amazonian soil conditions, as well as the effect on nutrition and fruit quality.** *Materials and methods* – **This study assessed a complete fertilization regime (N, P, K, Ca, Mg, S + lime), as well as the omission of nutrients to determine effects on fruit quality. Data was analyzed using multivariate statistics (main component analysis) by R 3.2.4 statistical software and the responses were examined using a statistical model run in SAS 9.3 software.** *Results and discussion* – **We found the highest flavor relation with the full treatment (all macronutrients) followed by the full treatment minus potassium (-K), this occurred because the effect of combined macronutrients increased soluble solid content. It was observed that the complete treatment produced lower acidity and higher soluble solids content in the naranjilla fruit, thus improving its quality.** *Conclusion* – **The quality of naranjilla fruit improved when the plant was provided with all the nutrients (N, P, K, Ca, Mg and S) plus lime because fruit acidity was reduced and there was increased soluble solid content and, consequently, enhanced flavor. Lime contributed to improve nutrient availability, especially in acid soils, which are characteristic of the Amazon region.**

Keywords
acid soils, Amazon, nutrients, lime

Significance of this study

What is already known on this subject?

- Naranjilla (*Solanum quitoense* Lam.) is an exotic tropical fruit of economic importance in the Andean region of South America and is also grown in Central America; it is appreciated in the local market and has export potential. There is few information about the main pest affecting this fruit crop, and some work related to breeding about crosses is carried out to generate new plant material.

What are the new findings?

- This study has generated new information that will contribute for the agronomical management of the fruit crop, in terms of fertilization. Plants that received all nutrients (N, P, K, Ca, Mg and S) plus lime improved the fruit's taste by reducing its acidity and increasing the soluble solids content. The lime-free treatments showed more availability of Zn, Cu, and Mn which are present in the acidic soils of the Amazon region. This information will allow to build fertilization programs focused in the main nutrients demanded by this fruit crop.

What is the expected impact on horticulture?

- Due to the lack of information about the fertilization of naranjilla, the results of this research will constitute a reference for the nutritional management of this crop, influencing positively the fruit quality. In addition, further studies on mineral plant nutrition could be done, taking into consideration our findings.

Introduction

Naranjilla or lulo (*Solanum quitoense* Lam.) is a fruit native to the Andes that is extensively cultivated and consumed in Ecuador, Colombia and countries of Central America (Costa Rica, Guatemala and Panama) (Acosta *et al.*, 2009; Lim, 2013). It is an important source of proteins, vitamins and minerals (González *et al.*, 2014). The fruit is a yellow, round oval berry, 2 to 6 cm in diameter, covered by small spines, with green pulp. It is aromatic, has a sweet and sour taste, and is high in vitamin A, C, B1, B2, proteins and minerals. The fruit is usually used to make juices, jams, jellies, desserts, nectars, sauces, dressings and cocktails (Andrade *et al.*, 2015; González *et al.*, 2014).

In Ecuador, the cultivated area is ca. 2,342 ha and the average yield is 6.04 t ha^{-1} and it grows in some low areas of the Highlands (valleys) and the Amazon (Ministerio de Agricultura y Ganadería, 2017; Vargas-Tierras *et al.*, 2018). The hybrid cultivar Puyo (*Solanum sessiflorum* × *Solanum quitoense* var. Quitoense) and the juice species (*Solanum quitoense* Lam.) of naranjilla are the most important variety and species in the country. 'Puyo' is characterized by having thorns on the leaves and stems, while the juice species of naranjilla has no thorns and the fruit is less acid than 'Puyo' (Andrade *et al.*, 2015; Silva *et al.*, 2016).

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The production of naranjilla in the Ecuadorian Amazon is concentrated in Napo, Pastaza, Morona Santiago, Sucumbios, Zamora Chinchipe, and Orellana, areas characterized by favorable agro-ecological conditions for the production of *S. quitoense* (Andrade *et al*., 2015). However, plant production depends on soil, climate, agronomic management and nutrition, among other factors that determine the quality and quantity of the crop (Almanza *et al*., 2008; Varela *et al*., 2001).

The quality of the fruit is assessed mainly by the appearance of the fruit: fruit size, color, firmness; harvest quality: respiration of the harvested fruit and storage capacity; and the nutrient composition: soluble solids content, acidity, vitamin content and other chemical and biochemical factors (Mancera-López *et al*., 2007; Medina *et al*., 2009).

The nutrients most highly related to the quality of the fruit are: potassium (K) and calcium (Ca), which are required in the development phase of the fruit and in biochemical and physiological processes, such as quality, maturation, color and yield (Rubio *et al*., 2010; Sedano *et al*., 2011). On the other hand, excess Ca has been reported to be responsible for softening and apical rot (Ho *et al*., 1999).

Magnesium (Mg) is important in fruit development and formation of fructose (Martínez *et al*., 2008). Phosphorus (P) is required in almost all growth processes, the synthesis of constituent compounds and for seed formation; in deficient conditions the maturity of the fruit is delayed (Alejo-Santiago *et al*., 2015).

While nitrogen is important in the growth and development of the leaves, it is also essential for cellular components (amino acids, proteins and nucleic acids) and as a regulator of P, K and other nutrients, so the effect on fruit growth would be indirect (Alejo-Santiago *et al*., 2015; Sedano *et al*., 2011). The application of optimal levels of N is required for improved organoleptic quality and vitamin C and soluble solids content (Hernández *et al*., 2002). Our hypothesis is that naranjilla plants that receive complete fertilization (all nutrients) according to the crop requirement plus an edaphic amendment will positively influence fruit quality parameters.

The objective of this study was to evaluate the effect on the quality of naranjilla fruit of the omission of one of the main macronutrients (N, P, K, Ca, Mg or S) from a full treatment package (N, P, K, Ca, Mg, S + lime); and a control treatment with no nutrients, with (C+lime) and without lime (C-lime).

Materials and methods

Fertilization practices

Three environments (locations) were selected for this experiment: Murialdo, 16 de Agosto, and Granja Experimental Palora (GEP). Murialdo is located in Fatima parish, province of Pastaza, located at 1,000 m a.s.l. (long. 9842124; lat. 0170217), whereas 16 de Agosto and GEP are located in Palora county, province of Morona Santiago, at 873 m a.s.l. The coordinates for 16 de Agosto are long. 09806781 and lat. 0175267, and for the GEP are long. 09815162 and lat. 0170283, respectively. Naranjilla variety INIAP Quitoense-2009 was used for this study in all environments.

From transplant to one year old, plants were fertilized with all the macronutrients except one that was omitted in the following manner: a full treatment with all macronutrients (N, P, K, Ca, Mg and S), a full treatment without nitrogen (-N), a full treatment without phosphorus (-P), a full treatment without potassium (-K), a full treatment without calcium (-Ca), a full treatment without magnesium (-Mg), a full treatment without sulphur (-S), no nutrients applied plus lime (C+ lime), and no nutrients applied without lime (C- lime). The nutrient sources used in this experiment were ammonium nitrate (38% N), Maistar calcium (40% Ca), di-ammonium phosphate (18% N, 48% P), calcium nitrate (12.3% N, 16.8% Ca), potassium sulfate (46% S, 51% K_2O), Magnesil (19.3% Mg), magnesium sulfate (12% Mg, 20% S) and KCl (60% K).

A randomized complete block design with three repetitions was used. The treatments were applied following fertilizer recommendations (Revelo *et al*., 2010), N was applied in five fractions, 20% at transplant and when plants were at three, six, seven and a half, and ten months of age. The K was divided into three lots: 50% at transplant, 25% at four months and the other 25% at eight months. Finally the P, Ca, S, Mg, were divided into two 50% lots, the first applied at transplant and the other half after six months.

The parameters taken into account for the analysis were: flavor relation, brix degrees, moisture (dry basis), titratable acidity, pH and the content of the following elements in the fruit: P, Ca, Mn, Zn, Cu, Mg, Fe, Na and K.

To perform the quality analysis, naranjilla fruit was harvested from the six plants of the experimental unit and three fruits were randomly selected by repetition, in stage 4 of maturation (full maturity) (Instituto Ecuatoriano de Normalización, 2009). Three fruits for each treatment and repetition were selected and the juice was extracted with a conventional blender. The soluble solids were determined in a manual refractometer (HANNA HI 96801). The ionic acidity (pH), was measured with a BOECO PT-380 potentiometer, the titratable acidity was determined by titration of 0.1% NaOH in the mixture of 10 mL of juice with 200 mL of distilled water, at pH 8.2. The nitrogen content was determined by the Kjeldahl method, phosphorus by UV/vis spectrophotometry and P, Ca and Mg by atomic absorption spectrophotometry.

Data analysis

1. Multivariate statistics. Multivariate analysis was performed using R 3.2.4 coupled with the MDA tools package. A principal component analysis (PCA) was used for unsupervised pattern recognition to determine associations between fertilization practices and fruit quality parameters at each location (environment). The following variables were taken into consideration: flavor relation, brix degrees, moisture (dry basis), titratable acidity, pH and the content of the following elements in the fruit: P, Ca, Mn, Zn, Cu, Mg, Fe, Na and K. The variables related to a given management practice were selected for univariate analysis.

2. Response analysis. The responses identified by PCA due to different fertilization practices were modeled as:

$$y_{ijkl} = \mu + L_i + B_{(i)j} + T_k + LT_{ik} + \varepsilon_{(ijk)l} \quad (1)$$

3 locations = i = 1,2,3 3 blocks j = 1,2,3
9 fertilization practices = k = 1 to 9; where:

y_{ijk} = denotes the observation at the i^{th} location in the j^{th} block for the k^{th} fertilization practice;
μ = grand mean;
L_i = fixed effect of the i^{th} location;
B_j = random effect of the j^{th} block in the i^{th} location NID $(0, \sigma^2_b)$;
T_j = fixed effect of the j^{th} fertilization practice;
LT_{ij} = interaction effect between the i^{th} location and the j^{th} fertilization practice;
$E_{(ijk)l}$ = random experimental error $(0, \sigma^2_e)$.

Table 1. Main effects and interactions for titratable acidity, flavor relation and Cu, Mn and Zn content of the fruit.

	Flavor relation	Titratable acidity	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
Environment (E)	NS	NS	NS	NS	**
Fertilization practice (T)	S	***	***	***	***
T × E	NS	**	**	NS	*

NS: not significant; S: significant at $p \leq 0.1$; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

Table 2. Cumulative variance explained by components in the three environments of this study. The variance explained by each component and the cumulative variance are presented. With five components around 75% of the variance is explained in all environments.

Component	16 de Agosto		GEP		Murialdo	
	Explained variance	Cumulative variance	Explained variance	Cumulative variance	Explained variance	Cumulative variance
Component 1	23.31	23.31	23.61	23.61	25.46	25.46
Component 2	16.16	39.46	20.22	43.83	22.14	47.60
Component 3	13.03	52.49	13.39	57.22	11.22	58.82
Component 4	11.86	64.35	10.60	67.82	9.68	68.50
Component 5	10.94	75.29	7.62	75.44	7.99	76.49

Table 3. Main effects of quality characteristics on naranjilla fruit for this experiment. Concentration in fruit of Mn (mg kg^{-1}), Cu and Zn (mg kg^{-1}), as well as flavor relation and titratable acidity determined for each factor: environment and fertilization practice.

Environment	Fertilization practice	Flavor relation	Titratable acidity	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
16 de Agosto		4.57 a	1.96 a	10.37 a	30.63 a	20.20 ab
GEP		4.55 a	1.94 a	12.87 a	28.26 a	19.04 b
Murialdo		4.36 a	1.96 a	15.18 a	28.23 a	21.67 a
	Full	5.28 a	1.63 d	6.38 d	22.74 c	14.87 e
	(-K)	4.86 ab	1.83 bcd	8.07 cd	23.05 c	15.66 de
	(-P)	4.53 abcde	2.10 abc	20.88 a	34.28 ab	25.98 ab
	(-S)	4.14 cde	2.02 abc	11.44 b	26.71 c	18.26 bc
	(-Mg)	4.00 e	2.20 a	11.23 bc	34.68 a	21.84 bc
	(-N)	4.65 abcd	1.81 cd	15.09 ab	32.95 ab	20.46 c
	(-Ca)	4.39 bcde	1.93 abcd	15.56 ab	29.80 bc	20.15 cd
	C+lime	4.74 abc	1.91 abcd	12.63 b	26.41 c	18.8 cd
	C-lime	4.02 de	2.19 ab	20.31 a	35.94 a	30.02 a

Mean values are reported. Within a column and within a given factor, means followed by the same letter are not statistically different (α = 0.1).

The data was analyzed using SAS 9.3 mixed model procedure. Interactions and main effects were declared significant at $p < 0.1$. Variables were checked for assumptions of normality and homogeneity of variances based on a plot of residuals *vs.* predicted values. Transformations were performed as needed to comply with the assumption of normality. The transformations were based on the Box-Cox power transformation series (Box and Cox, 1964). Least square means were separated using LSD mean separation procedure in SAS proc mixed. The LSD differences were reported at P = 0.1 (Saxton, 1998).

Results

PCA modelling

1. Environment 16 de Agosto. The data were mean centered and standardized prior to PCA modeling. A non-parametric Spearman test was performed previous to PCA modeling and showed that environment, repetition and fertilization practice were not correlated. Scaling was performed since the elements were expressed in different units. The final model included eight principal components (Table 1); however, for this experiment, only five components were necessary to explain the main structural information (cumulative explained variance ~75%) (Table 2), while the others were mostly responsible for a random variation.

The first two components show that titratable acidity, Zn and Cu contents were higher in fruit when lime was not applied (C-lime). The flavor relation in fruit was higher in fruit with the full treatment. The fertilization practice (-K) was characterized by higher pH and higher Fe content (Figure 1).

The first component separated the effect of the control without lime and the second the effect the full treatment. For instance fruit from C-lime were characterized by a higher content of Zn (between 38 and 56 mg kg^{-1}), Cu (> 38 mg kg^{-1}) and titratable acidity (> 2.09). The second component had

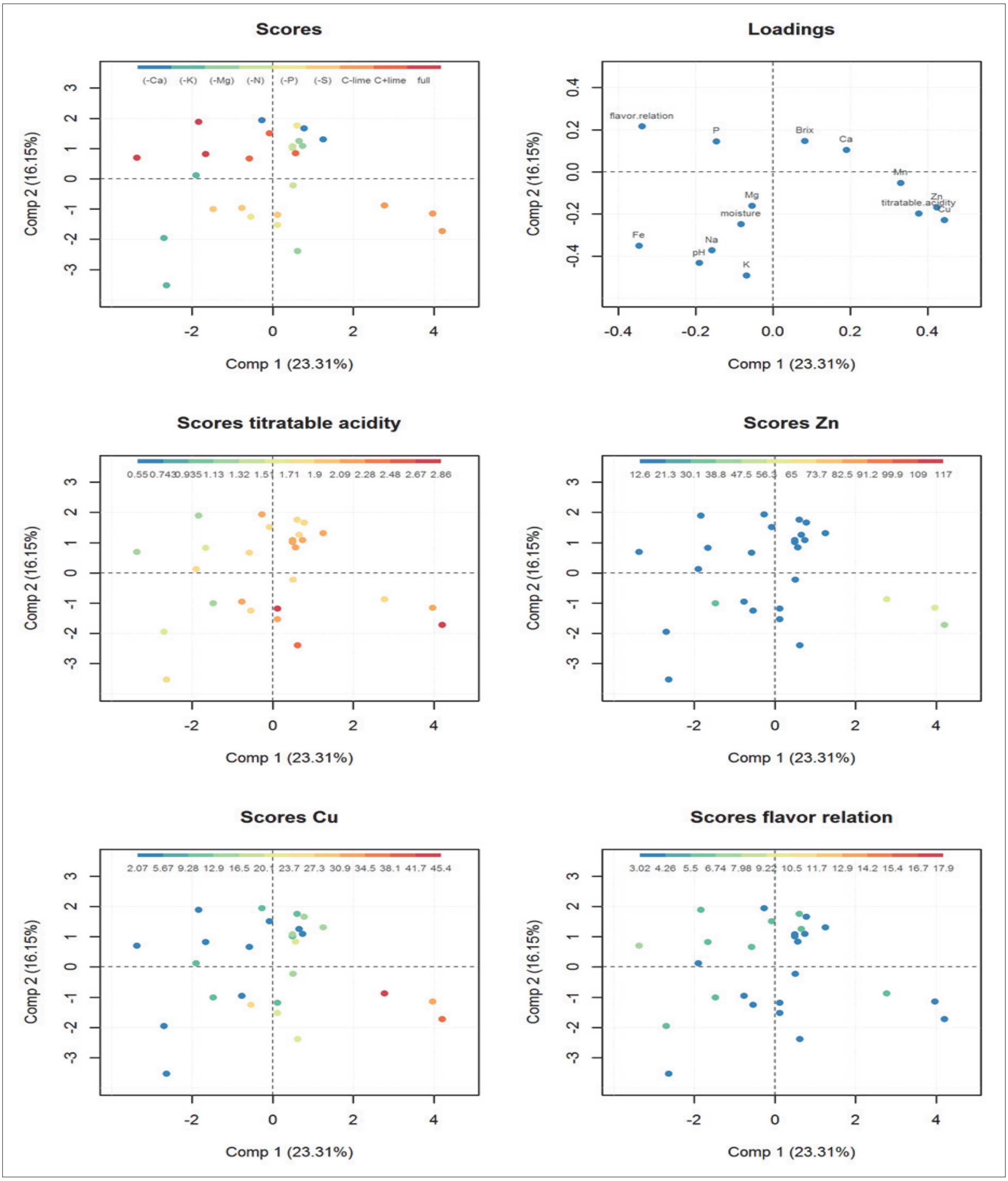

Figure 1. Principal component analysis on naranjilla fruit at 16 de Agosto. Panel B is the loading plot and panels A to F the score plots. The principal component 1 and 2 are plotted in panel A and B showing the fertilization practices and the corresponding variables. Panels C to F provide a representation of the score plot colored with respect to the amount of a given variable (from low to high).

less clear separation between fertilization practices and the full treatment (all fertilization practices) has some increase in the amount of flavor relation (Figure 1). The other components did not show any discernable pattern.

2. Environment GEP. As with 16 de Agosto, a PCA model was performed on data from the environment GEP (Figure 2). The final model included seven principal components, but five explain approximately 75% of the variance (Table 3). There was not a clear pattern as with the 16 de Agosto environment; however, the practice of C-lime had much higher Cu, Zn and Mn in one replicate. Mean values are reported. Within a column and within a given factor, means followed by the same letter are not statistically different ($\alpha = 0.1$).

3. Environment Murialdo. The final PCA model included seven principal components, but five explained approximately 76% of the variance, which is similar to the other en-

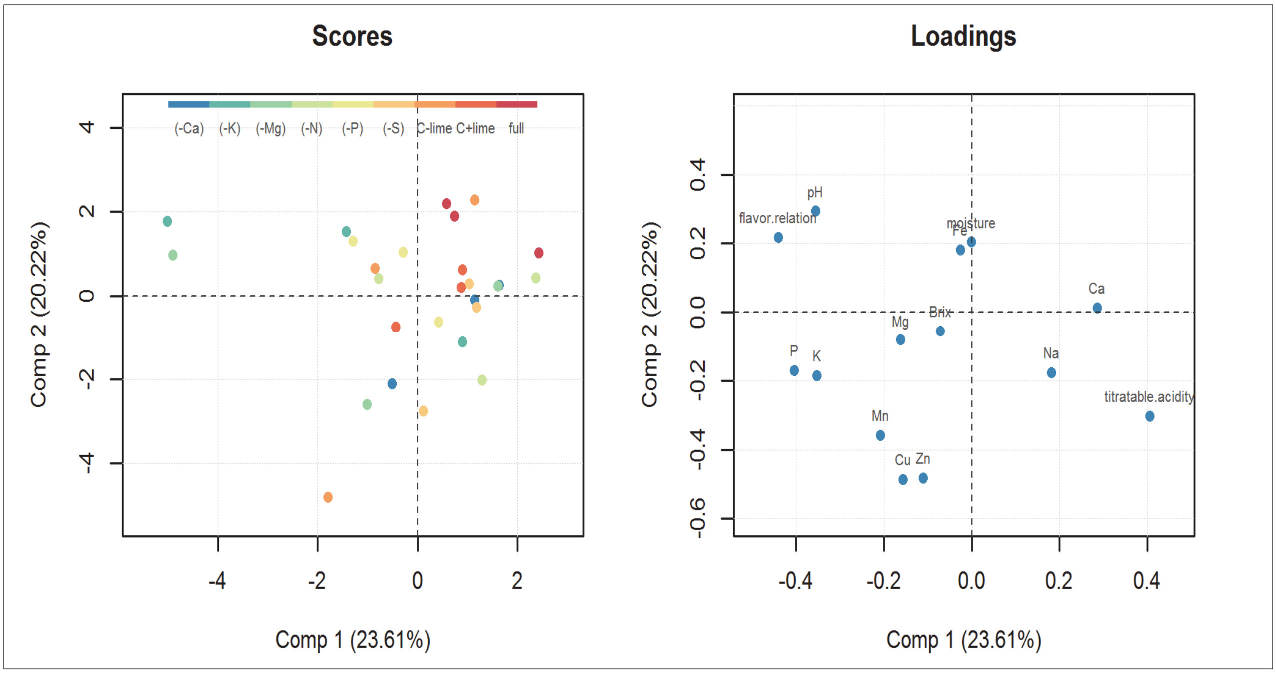

FIGURE 2. Principal component analysis of naranjilla fruit in the GEP environment. Panel A is the score plot and panel B is the loading plot.

TABLE 4. Interaction effects of environment and fertilization practice on the quality characteristics of naranjilla. The Cu and Zn concentrations (mg kg^{-1}) in fruit and titratable acidity were determined for each factor: environment and fertilization practice.

Environment	Fertilization practices	Titratable acidity	Cu (mg kg^{-1})	Zn (mg kg^{-1})
16 de Agosto	Full	1.43h	3.01i	15.67i
	(-Ca)	1.99abcdef	13.38cdefg	22.06bcdef
	(-K)	1.80bcdefgh	5.83h	15.76hi
	(-Mg)	2.16abcde	8.83efgh	18.29bcdefgh
	(-N)	2.07abcdef	14.51cdef	19.93bcdefg
	(-P)	1.96abcdef	16.89cd	20.51bcdefg
	(-S)	1.95abcdefg	7.58gh	17.97efgh
	C+lime	1.97abcdef	9.59defgh	18.14cdefgh
	C-lime	2.26ab	37.51ab	54.23a
GEP	Full	1.70efgh	10.17cdefg	13.37b
	(-Ca)	2.09abcdef	19.09bc	23.82abcde
	(-K)	1.54gh	11.34cdefg	16.01fghi
	(-Mg)	2.25abc	9.43defgh	18.97bcdefgh
	(-N)	1.76cdefgh	15.77cde	18.03defghi
	(-P)	2.35a	13.51cdefg	19.58bcdefgh
	(-S)	2.05abcdef	15.41cdef	16.80efghi
	C+lime	2.02abcdef	12.42cdefg	24.51abcd
	C-lime	1.99abcdef	11.41cdefg	25.71abcd
Murialdo	Full	1.78bcdefgh	8.46efgh	15.77ghi
	(-Ca)	1.73defgh	14.75cde	18.53bcdefgh
	(-K)	2.23abcd	7.94fgh	15.21j
	(-Mg)	2.20abcd	17.00cd	31.31abc
	(-N)	1.64fgh	15.02cde	24.08abcd
	(-P)	2.02abcdef	39.91a	49.80ab
	(-S)	2.07abcdef	12.80cdefg	20.25bcdefg
	C+lime	1.75defgh	16.91cd	18.66bcdefgh
	C-lime	2.34ab	19.58bc	21.73bcdefg

Mean values are reported. Within a column and within a given factor or combination of factors, means followed by the same letter are not statistically different according to the LSD test (α = 0.1). Comparisons are within environments.

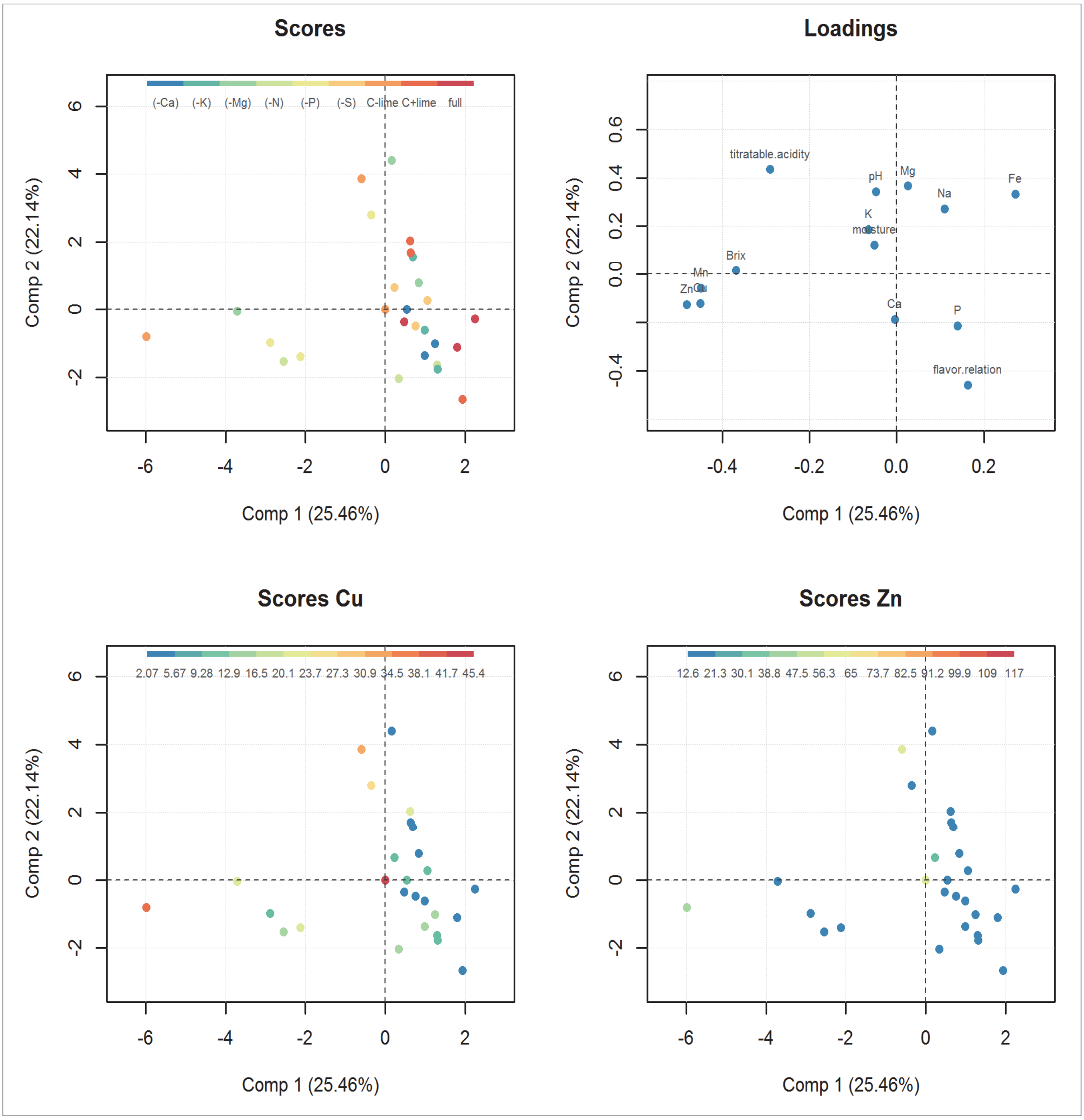

FIGURE 3. Principal component analysis of naranjilla fruit at Murialdo. Panel A is the score plot and panel B the loading plot. Panels C and D provide a visualization of the score plots colored with respect to the concentration of Cu and Zn.

vironments (Table 2). As at GEP, at Murialdo (Figure 3) there was no clear pattern in the PCA from which to understand the practices that influence the fruit the most. As at GEP, the fertilization practice C-lime had much higher Cu, Zn and Mn in one of the replicates.

Univariate analysis

Univariate analysis was performed for the following variables: titratable acidity, flavor relation, Cu, Mn and Zn. Those variables were somewhat influenced by the practices used as modelled by PCA. The main effects and interactions between the locations and the fertilization practices were analyzed as well (Tables 3 and 4). The analysis showed that in general there were no differences among practices due to environment, except for Cu ($p=0.005$) and Zn ($p=0.0030$), where there was an interaction between environment and practice. The influence of fertilization practice was significant in all environments. The highest flavor relation was found with the full fertilization practice, followed by (-K) (Table 3). Several fertilization practices caused higher titratable acidity, but the lowest acidity was observed for the full treatment.

The interaction between fertilization practices and environment was only significant for titratable acidity, Cu and Zn. At 16 de Agosto, we found the lowest titratable acidity with the full fertilization treatment, which was detected by the PCA analysis as well (Figure 1). The highest Cu and Zn contents were observed at 16 de Agosto combined with the C-lime treatment and the lowest with the (-K) and full treatments (Table 4). The treatments without Mg, N, or S resulted in approximately the same level of acidity as the Cu and Zn treatments, regardless of the environment. In the other locations higher Cu content was found with (-Ca) at GEP and (-P) at Murialdo.

Discussion

Previous studies on *S. quitoense* have used univariate statistics to discriminate the variables and determine causation based on practices. In this study we analyzed an array of variables to find correlations between the variables and the practices. We found the highest flavor relation with the full treatment (all macronutrients) followed by the full treatment minus potassium (-K), this occurred because of the effect of combined macronutrients increased soluble solid content as reported previously (Valverde *et al*., 2009).

It was reported that *Solanum lycopersicum*, which belongs to the same genus as *S. quitoense*, had increased titratable acidity and maintained soluble solid content under the highest amount of potassium fertilization; however, the flavor ratio decreased (Oliveira *et al*., 2015; Wuzhong, 2002). In another solanaceous species (*Capsicum annuum*), the same authors found that fertilization with potassium increased soluble solid content, thus elevating the flavor ratio. In this study of *S. quitoense*, the complete treatment (all minerals + lime) increased the soluble solid content of the fruit, hence the flavor ratio was also augmented. Potassium favors the translocation of photosynthates (including sugars) from the leaves to the fruit (Espinoza *et al*., 2017); developing fruit requires 70 and 80% of K as nutrient, especially for uniform ripening, accumulation of organic acid to improve flavor and to encourage uptake of water to the fruit (Montoya *et al*., 2002).

In addition, the flavor of the fruit increases when S, N and K are available (Mattheis and Fellman, 1999), which is possibly achieved when lime is applied to the soil (Sainju *et al*., 2003). Calcium and Mg are important for fruit quality and are released when they are added to limed soil.

We found that the Zn and Cu content was higher in fruit in the control without lime (C-lime). The accumulation of Zn in fruit was due to the fact that pH is the main factor that determines availability of this element in the soil; low pH increases Zn solubility in acid soils (pH from 4 to 5.5), which is characteristic of the Amazon region. The pH of the soil solution is a good indicator of the availability of nutrients (Madrigal-Soteno *et al*., 2016; Osorio, 2012); and Mn, Zn, Cu and Fe become readily available under acid soil conditions, as under the conditions of this experiment. Other authors have found that Mn absorption in *S. quitoense* was higher than Zn, followed by Cd, Cu and Pb (Méndez *et al*., 2009).

We found that the levels of Cu, Mn and Zn in the full treatment were 6.38, 22.74 and 14.87 mg kg^{-1}, respectively. However, for the control-lime treatment the levels of Cu, Mn and Zn were 20.31, 35.94 and 30.02 mg kg^{-1}, respectively; therefore it was inferred that the application of lime influences the translocation of these three minerals to the fruit.

The European Union indicated that the maximum Cu and Zn contents in the fruit should be maintained between 10 and 25 mg kg^{-1}. Therefore, the amounts of these elements in the fruit from the full treatment (all minerals + lime) are within allowable limits.

Several studies have reported variation in Zn content in the fruit depending on the species studied. In this study, the amount of Zn in the fruit of *S. quitoense* was in the range of 14 to 30 mg kg^{-1}, a value higher than that reported previously (5 mg kg^{-1}) (Ali and Al-Qahtani, 2012). On the other hand, similar values were reported in studies conducted on *Vaccinium myrtillus*, *Vitis vinifera*, *Rubus idaeus*, *Pyrus malus*, *Prunus armeniaca* and *Prunus domestica* (15 to 60 mg kg^{-1}) (Noulas *et al*., 2018).

Conclusions

The quality of naranjilla fruit improved when the plant was provided with all the nutrients (N, P, K, Ca, Mg and S) plus lime because fruit acidity was reduced and there was increased soluble solid content and consequently, enhanced flavor. Lime contributed to improve nutrient availability, especially in acid soils, which are characteristic of the Amazon region. However, there was greater accumulation of Zn, Cu and Mn in the fruit corresponding to the treatment without lime because in acidic soils these elements become more available in the soil solution, improving assimilation in the plant.

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