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Seasonal Changes and Nutrient Concentrations of "Cristalina" Cactus Pear Cladodes in Response to NPK Fertilization

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ABSTRACT
There are scarce and conflicting reports on fruit yield (FY) and cladode nutrient concentrations for cactus pears (*Opuntia* spp.). The objective of this study was to examine the macro- and micronutrient cladode concentrations, the association between FY nutrient concentrations, and seasonal changes in macro- and micronutrients in fruiting cladodes of "Cristalina" cactus pear plants exposed to a nitrogen (N), phosphorus (P), and potassium (K) fertilization matrix over three growing seasons. The experiment was conducted in a randomized complete block design under field conditions. Seven samples of cladode were collected at different phenological stages for the measurement of N, P, K, calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), and boron (B). The analysis suggested that the most important nutrients for FY (20.5 t ha^{-1}) were N and P at 90 and 30 kg ha^{-1}, respectively. The seasonal pattern for macro- and micronutrients is shown and discussed. The latest topic deserves further research for understanding the cactus pear ionome and suggesting mineral fertilization programs to the cactus pear growers.

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KEYWORDS
Mineral nutrition; nitrogen; *Opuntia albicarpa* Scheinvar; phosphorus; potassium

Introduction

Cactus pear (*Opuntia* spp.) is an important Mexican fruit crop that is now grown commercially in many countries of the Mediterranean basin (Arba 1996; Inglese et al. 2010; Mimouni, Ait Lhaj, and Ghazi 2013), Argentine (Targa et al. 2013), Chile (Mora et al. 2013), South Africa (Shongwe et al. 2013), and the United States of America (Felker and Bunch 2009). This xerophitic plant is cultivated in marginal arid and semiarid lands where water for irrigation is a limiting factor (Nefzaoui and Ben Salem 2002; Nobel 1994). Nevertheless, in Mexico 1.3% of the land (ca 55 000 ha) cultivated with cactus pear is irrigated (SIAP, 2016). Therefore, fruit yield (FY) can be increased by using irrigation (García De Cortázar and Nobel 1992; Gugliuzza, Inglese, and Farina 2002a; Nerd, Karady, and Mizrahi 1989) and mineral and organic fertilization (Nerd, Karady, and Mizrahi 1989, 1991; Pimienta-Barrios and Ramírez-Hernández 1999; Zegbe, Serna-Pérez, and Mena-Covarrubias 2014).

Continuous irrigation and mineral fertilization promoted not only early flower bud emergence, but also high concentrations of nitric nitrogen and potassium (K) in cladodes of "Ofer" cactus pear (Nerd, Karady, and Mizrahi 1991). Both reproductive buds and nitrogen (N) concentration in current cladodes of "Ofer" cactus pear were enhanced by applying 120 kg N ha^{-1} (Nerd and Mizrahi 1994). As in deciduous fruit trees (Westwood 1993), tissue nutrient status is important for cactus pear plant performance. Early research by Gathaara, Felker, and Land (1989) revealed that whole-plant N and K cladode tissue concentrations correlated positively with FY. Cladode tissue concentrations of 1.16% N and 0.115% phosphorous (P) produced the maximum fruit number (≈ eight fruits per cladode) in

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Opuntia engelmannii. Later on, Karim, Felker, and Bingham (1998) found that 1.37% N and 11% K cladode tissue concentrations, at the whole plant level, correlated positively with FY, while the sodium (Na) tissue concentration correlated negatively. However, fruit sugar correlated positively with magnesium (Mg) tissue concentration. The maximum fruit sugar concentration (≈12%) was found at cladode tissue concentration of 2.2% Mg. The same authors concluded that cladode K tissue concentration correlated negatively with fruit number in two of five wild *Opuntia* accessions. These studies provide more discrepancies than coincidences, which could be due to the different *Opuntia* germplasms used, plant phenological stages, and cladode tissue sampling dates. It is desirable to learn more about seasonal changes in cladode macro- and micronutrient concentrations and their relation to FY since, so far, there is just one previously published report on this topic (Gugliuzza, La Mantia, and Inglese 2002b). The latter authors found that the fruiting cladodes of "Gialla" had a significant reduction in N and K concentrations through the growing season, while the opposite was observed for Mg and calcium (Ca) concentrations. However, seasonal cladode tissue concentrations of P and micronutrients were not reported.

The objective of this study was to examine macro- and micronutrient cladode concentrations, the association between FY and nutrient concentrations, and seasonal changes in macro- and micronutrients in fruiting cladodes of "Cristalina" cactus pear plants exposed to an NPK fertilization matrix over three consecutive growing seasons. We measured phenological and seasonal changes in the tissue concentrations of 10 nutrients in response to several mineral nutrient treatments (MNTs). Additionally, nutrient tissue concentrations and FY were used to identify the best MNT.

Materials and methods

Experimental site

The experiment was performed in a commercial orchard in Santa Fe, Jerez, Zacatecas, Mexico (lat. 22°32′N, long. 103°03′W, elevation 1,976 m). The trial was conducted for three successive growing seasons (2004 to 2006) in a semiarid area.

The site has an annual mean temperature of 25.7 °C, 482 mm annual precipitation, 62% of which occurs between July and October, and 2,245 mm annual evaporation. The soil is classified as Fluvisol with a sandy-loam to loam texture, pH from 6.1 to 7.4, and organic matter between 1% and 1.56%. Analysis of soil fertility prior to the application of fertilizer treatments revealed a total concentration (mg kg^{-1}) of 0.71 inorganic N, 0.1 total N, 2.0–3.2 P, 520–1,173 K, 1,834–4,440 Ca, and 213–334 Mg.

Plant material and orchard management

Four-year-old cactus pear plants of "Cristalina" (*Opuntia albicarpa* Scheinvar) were used. This variety is white-pulped and late-maturing. Plants were trained to an open vase system and spaced at 4 m between rows and 3 m within rows. Except for fertilization, plants were handled for commercial production including cladode pruning, fruit thinning, pest and weed control as needed, and row irrigation. Irrigation was applied following the commercial practice based on meteorological data collected from an automated weather station placed 2.5 km away from the orchard.

MNTs and experimental design

The mineral nutrition supplements were N, P, and K. The sources of NPK were urea (46% N), triple superphosphate (46% P_2O_5), and potassium chloride (60% K_2O), respectively. Four rates each of N and P and three rates of K were arranged in an incomplete factorial matrix (Table 1). The 10 MNT were randomized in three blocks. Each experimental unit comprised five consecutive plants in a row with three unfertilized plants at each end surrounding the experimental units.

Table 1. Mineral nutrition treatments applied to "Cristalina" cactus pear plants for three consecutive growing seasons from 2004 to 2006 in Jerez, Zacatecas, Mexico.

	Mineral nutrient rates (kg ha^{-1})		
Treatment	Nitrogen	Phosphorus	Potassium
1	00	00	00
2	00	30	30
3	30	30	30
4	60	30	30
5	90	30	30
6	60	45	30
7	60	60	30
8	60	30	00
9	60	30	60
10	90	60	60

Fertilization program

For each fertilization treatment, half of the N and all of the P and K were manually incorporated at a soil depth of ≈5 cm, and around and 50 cm away from the plants at the start of bloom on 6 April 2004, 19 April 2005, and 21 April 2006. The remaining half of the N was applied two to three weeks after fruit harvest. The second N applications were carried out manually on 19 October 2004 and 2005 and on 22 September 2006.

Cladode sampling and nutrient analysis

Samples for determining macro- and micronutrient concentrations were taken of a one-year-old fruiting cladode from the central plant of each experimental plot using a coring device. The specimens included both clorenchyma and parenchyma from the apical side of each cladode. They were collected on 21 July (fruit elongation stage) in 2004; on 15 February (63 days before blooming), 2 June (half of the first fruit growth), 12 July (fruit elongation stage), and 12 September (at 50% of harvest) in 2005; and on 1 March (51 days before blooming) and 28 April (seven days before blooming) in 2006. Samples preparation and determination of nutrient concentrations were described by Zegbe, Serna-Pérez, and Mena-Covarrubias (2014).

FY determination

Fruits were manually collected at export harvest maturity skin color (yellowish-green) from the two central plants of each experimental plot on 30 August 2004, 24 August 2005, and 18 July 2006.

Data analysis

The data were analyzed in randomized complete block model using the GLM procedure of SAS (version 9.2; SAS Institute, Cary, NC). Although using an incomplete factorial matrix is a statistical strategy to reduce the experiment dimension, this statistical tool does not allow testing the null hypothesis over the interaction among factor levels. Data were also examined using canonical discriminant analysis (CDA) to provide an overview of the FY and macro- and micronutrient concentrations all together and to identify major sources of difference among MNTs. This was done using the CANDISC procedure of SAS. Means of treatment and standardized canonical scores (from CDA) were ranked by Tukey's studentized range test at $p \leq 0.05$.

Results and discussion

Regardless of FY improvement, application of sodium chloride (NaCl) (Nerd, Karady, and Mizrahi 1991) or NPK fertilization to the growing medium produced measurable changes in the nutrient concentrations of cactus pear cladodes (Claassens and Wessels, 1997; Galizzi et al. 2004; Karim, Felker, and Bingham 1998; Nerd and Mizrahi 1994). One objective of our experiment was to detect significant changes in tissue nutrient concentrations after MNTs; however, MNTs produced no significant differences among nutrients on 21 July 2004. The same was true for all nutrients on the four sampling dates in 2005, except for N on the fourth sampling date (12 September) and B at the second sampling date (2 June). In 2006, there were no measurable changes in nutrient cladode concentrations on two sampling dates, except for N cladode concentration on the first sampling date (1 March). Based on these responses, we decided to bulk the information for each nutrient from the seven sampling dates. The latter analysis produced similar mean values for each nutrient (data not shown) to those reported already by Zegbe, Serna-Pérez, and Mena-Covarrubias (2014). Consequently, a CDA was conducted, considering collectively the FY and cladode concentrations of 10 mineral nutrients. This multivariate analysis revealed significant (p = 0.0274) differences among MNTs. The first two canonical variates (CVs) had the largest eigenvalues and accounted for 74% of the separation among MNTs, where only the first CV (CVI) was significant ($p < 0.05$). The second CV (CVII) was not significant, but was kept because of the relevance of cladode P concentration (Table 2). Therefore, the standardized canonical coefficients (SCC) of CVI suggest that the separation and grouping of MNTs accounted for a contrast between high yield and high cladode concentrations of N and Ca, but lower cladode concentrations of Zn and B. However, the correlation among original variables with the first CV confirmed that the separation among MNTs was weighed toward high FY and N cladode concentration only. In contrast, the correlation among original variables with CVII accounted for the separation among MNTs due to P concentrations only. These two results were corroborated by an analysis of variance for CVI and CVII (data not shown). The latter analysis formed three MNT groups where the best and the worst treatments were treatment five (90 N-30P-30 K) and the control (0 N-0P-0 K) and treatment two (0 N-30P-30 K), respectively. The remanding treatments had intermediate mean values of canonical scores (Figure 1).

As described previously, MNT did not induce significant differences in cladode macro- and micronutrients, except for boron (B) and N on the second and fourth sampling dates in 2005. Therefore, we plotted the mean values (±95% confidence intervals) of each nutrient in 2005 and 2006. Gugliuzza, La Mantia, and Inglese (2002b) reported seasonal changes in cladode concentrations of N, K, and Mg in "Gialla" cactus pears compared to crop load. These authors observed a

Table 2. Standardized canonical coefficients (SCC) and correlation (r) between the original variables with canonical variates (CV) of nutrient concentration and fruit yield in one-year-old fruiting cladodes of "Cristalina" cactus pear.

	CVI		CVII	
Response variables	R	SCC1	r	SCC2
Fruit yield	0.71	0.89	0.14	−0.03
Nitrogen	0.58	1.01	−0.10	−0.68
Phosphorus	0.32	0.12	0.74	1.12
Potassium	0.06	−0.28	−0.07	0.00
Calcium	−0.04	0.41	−0.16	−0.30
Magnesium	−0.03	−0.05	−0.12	−0.20
Iron	0.02	0.15	0.11	−0.03
Zinc	−0.03	−0.31	0.22	0.33
Manganese	0.15	−0.13	0.36	0.27
Cooper	0.02	0.00	0.08	−0.45
Boron	−0.04	−0.37	−0.01	0.05
Eigenvalue		1.2		0.6
Variance explained (%)		49		25
Significance (p > F)		0.03		0.69
Coefficient of determination (%)		52		36

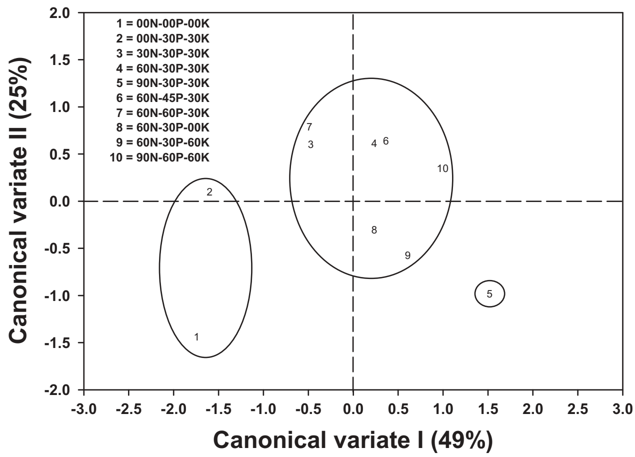

Figure 1. Mean canonical scores of the first two canonical variates for fruit yield and nutrients in one-year-old fruiting cladodes of "Cristalina" cactus pear given 10 mineral nutrition treatments.

significant depletion in N and K in the early stages of fruit growth, but both nutrients remained stable until harvest; however, the behavior of Mg was similar to that reported here. In 2005, N and zinc (Zn) concentrations decreased significantly from 15 February (63 days before blooming) to 12 July (fruit elongation); from the latter date the concentration of both nutrients increased significantly until 50% of fruit maturation. P, K, Ca, iron (Fe), and manganese (Mn) followed a similar pattern; however, the concentrations of these nutrients started to increase 40 days earlier (2 June) than the N and Zn concentrations (Figures 2 and 3). The seasonal pattern of cladode Mg concentration was atypical because it increased from 15 February to 2 June, and then decreased by 15 July. Finally, Mg cladode concentration recovered between fruit elongation (15 July) and fruit maturation (12 September; Figure 2E). In contrast, both copper (Cu) and B nutrient concentrations increased from 15 February (63 days before blooming) to 2 June (half of the first fruit growth), after which they decreased for the rest of the sampling dates (Figures 3D and 3E). In 2006, the first two of four cladode samples were used to determine nutrient cladode concentrations. The latter two samples were discarded due to fungal contamination. Based on the first two sampling dates, all cladode nutrient concentrations followed the same pattern at the start of the 2006 growing season except for Mg (Figure 2E). Unlike other fruits (e.g., peaches, apples, citrus, etc.; Lang and Thorpe 1986), fruits of *Opuntia* are attached directly (sessile fruit) to the source (cladodes) of nutrients and photo assimilates. Therefore, the strong depletion in N, P, K, Ca, Fe, Zn, and Mn from the first to second and third sampling dates is most likely due to the translocation of these nutrients from the cladode to the fruits during their growth. When the fruit matures, the demand for resources (nutrients and photosynthates) by the sink organs (fruit) ends; hence, the nutrient concentrations recover in the cladode tissues (chlorenchyma and parenchyma). On the other hand, Cu and B were transported into the fruit from the half of the first fruit growth and for the rest of the growing season. Both nutrients tended to recover by bloom of the next growing season.

After harvest, an end to cactus pear growth (ecodormancy) is imposed mainly by the low temperature, short photoperiod, and low or no water input occurring during autumn and winter. However, during cactus pear ecodormancy, photosynthesis and respiration continue at a low rate (Raveh and Nobel 1999). In 2006, the cladode P concentration was lower at the start of the 2006 growing season than in the previous growing season at fruit maturation (Figure 2B). The latter pattern could be due to the use of P (as $PO_4$3-) in the respiration process during ecodormancy. P was depleted along with N, Mg, Ca, Fe, Zn, and Mn during flower bud formation; however, these seasonal changes need further research.

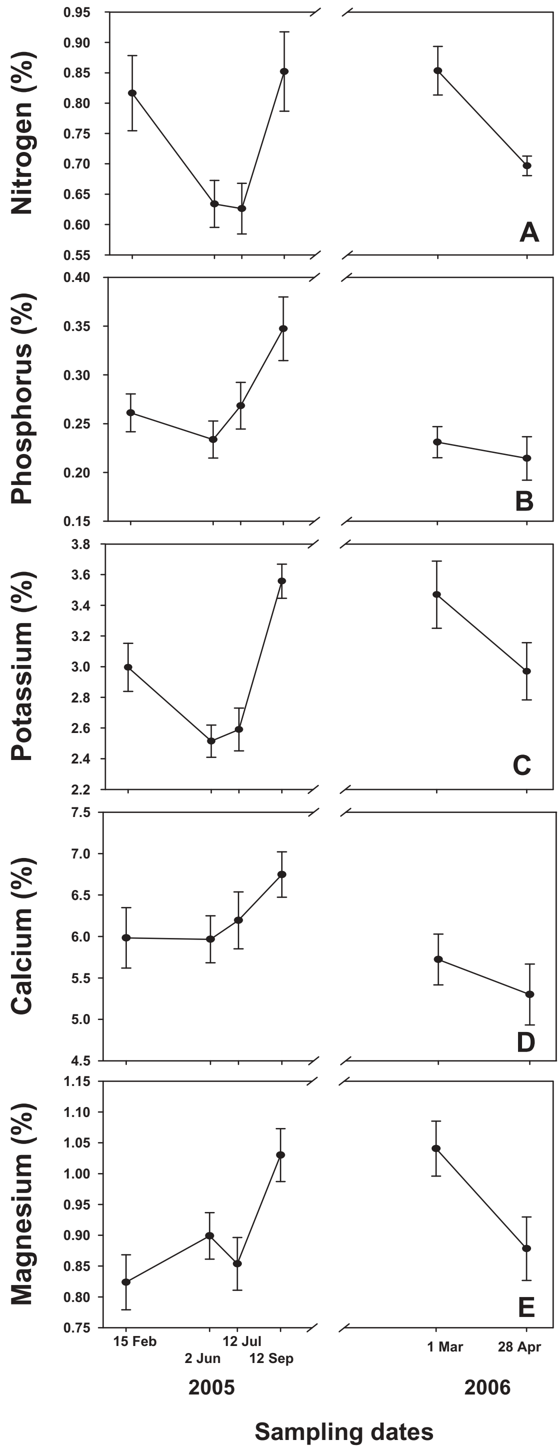

Figure 2. Seasonal changes in macronutrients in one-year-old fruiting cladodes of "Cristalina" cactus pear in Jerez, Zacatecas, Mexico. Vertical bars represent 95% confidence intervals at each sampling date (n = 30).

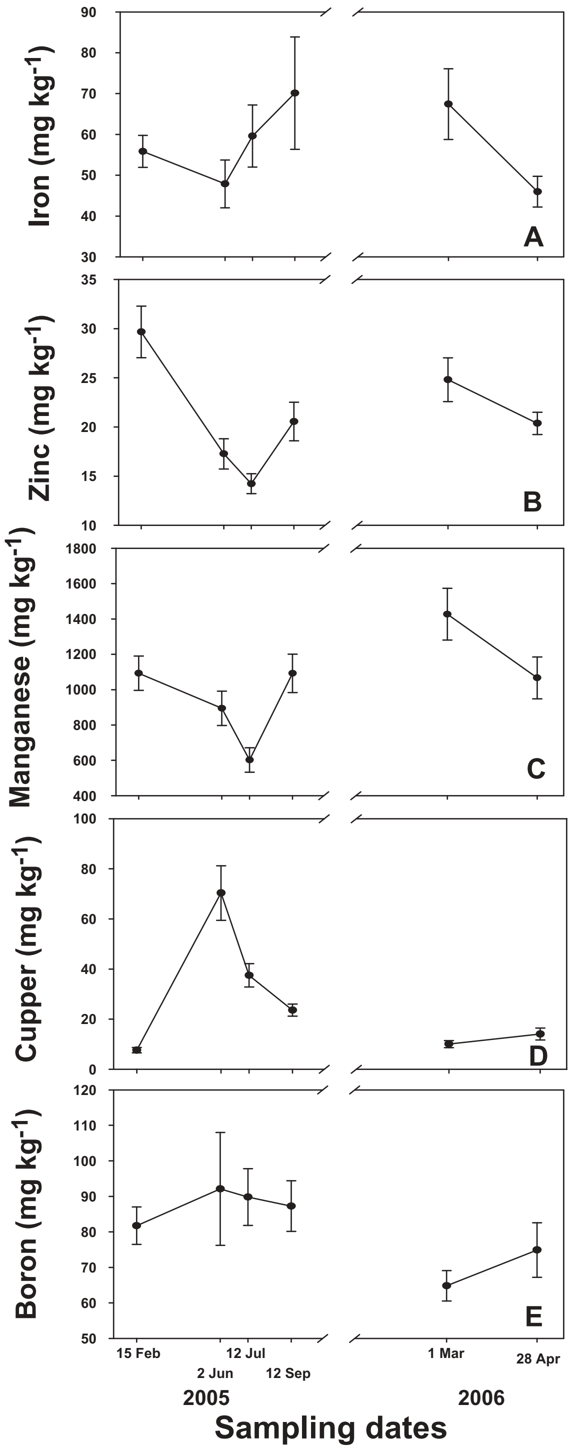

Figure 3. Seasonal changes in micronutrients in one-year-old fruiting cladodes of "Cristalina" cactus pear in Jerez, Zacatecas, Mexico. Vertical bars represent 95% confidence intervals at each sampling date (n = 30).

Conclusions

In summary, mineral fertilization had little or no effect on macro- and micronutrient cladode concentrations. CDA separated to the MNT 5 (90 N-30P-30 K) with both high FY (20.5 t ha^{-1}) and high cladode N (0.82%) concentration. The second group of MNTs (treatments 3, 4, 6, 7, 8, 9, and 10) had, on average, a cladode concentration of 0.81% N and 0.26% P, and 15.5 t ha^{-1} FY. The third group included the treatments 1 (0 N-0P-0 K) and 2 (0 N-30P-30 K) with the lowest N (0.64%) and P (0.17%) cladode concentrations and FY (6.7 t ha^{-1}). Fertilization with K was not important for either cladode nutrient concentrations or FY. In fact, the three groups had similar K cladode concentration as follows: 3.2%, 3.4%, and 3.3% for groups I, II, and III, respectively.

Regardless of mineral nutrient additions, cladode concentrations of N, P, K, Ca, Fe, Zn, and Mg had similar seasonal patterns. They were depleted during the first two-thirds of fruit growth and then recovered over the rest of the season. In contrast, we observed an accumulation of Cu and B in the cladodes during the first third of fruit growth; thereafter, these nutrients were depleted for the rest of the season. This is the first report offering a seasonal pattern of macro- and micronutrients in cactus pear cladode; however, the roles of Na, S, and Cl must also be defined to understand the cactus pear ionome.

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