

Short Research Article

Leaf Nutrient Concentrations and Dry Biomass of Fig plants as Modified by the Application of NPK: A Preliminary Study

ABSTRACT

Aims: The effect of a complete NPK matrix on leaf nutrient concentrations and dry biomass of 'Black Mission' fig plant organs ~~was~~ tested under an intensive culture system and protected environment.

Study design: A randomized complete block design with four blocks was employed.

Place and Duration of Study: The experiment was conducted from April to November 2016 at the Campo Experimental La Laguna, located in Matamoros, Coahuila, Mexico. This research station belongs to the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) of Mexico. The experiment was set up under a macro tunnel equipped with a shade mesh with 50% sunlight attenuation.

Methodology: Two-year-old fig plants (cv. 'Black Mission') previously propagated from stem cuttings were used. There were three application rates each for N (0, 80, and 160 kg ha⁻¹), P (0, 40 and 80 kg ha⁻¹), and K (0, 80, and 160 kg ha⁻¹) arranged in a balanced factorial matrix of treatments 27. After harvest, leaf samples were collected to determine nutrient concentrations and they were split into roots, shoots, leaves, and fruit

Results: The greatest total dry biomass was produced by the interaction of 80 kg ha⁻¹ N and 40 kg ha⁻¹ P and yielded the following leaf nutrient concentrations (mean ± SD): N 2.9 ± 0.3%, P 0.11 ± 0.01%, K 2.1 ± 0.4%, Ca 1.4 ± 0.7%, Mg 0.34 ± 0.03%, Fe 166.4 ± 49.5 mg kg⁻¹, Cu 6.3 ± 1.7 mg kg⁻¹, Mn 83.3 ± 20.9 mg kg⁻¹, and Zn 22.6 ± 3.8 mg kg⁻¹. Application of 80 kg ha⁻¹ N and 40 kg ha⁻¹ P could be suggested for commercial fig production.

Conclusion: Application of 80 kg ha⁻¹ N and 40 kg ha⁻¹ P could be tested under similar commercial production systems; however, the addition of supplemental K deserves further study.

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Keywords: *Ficus carica* L.; macronutrients; micronutrients; synergism-antagonism; nutrient use efficiency.

1. INTRODUCTION

The fig tree (*Ficus carica* L.) is one of the oldest crops in the Mediterranean basin, where its fruit is consumed either fresh or dry [1]. Fig fruits are an excellent source of nutrients and antioxidants for human health [2, 3] (Solomon et al., 2006; Sadia et al., 2014). In 2018, fresh fig production was 1.17 million tons worldwide. Turkey accounted for 26.2% of the total production followed by Egypt (15.2%), Morocco (11.8%), Algeria (11%), and Iran (6.1%). These five countries collectively yielded 70.2% of the total world production [4].

Fig, carob, olive, and almond trees are cultivated extensively in Mediterranean countries, typically without irrigation [1]. Fig tree hardiness has allowed it to be grown extensively in different soil types in warm climates [5]. This fruit crop has been gaining commercial interest around the world, and under intensive cultivation under soil-less and glasshouse culture yields reach from 81 t ha⁻¹ [6] up to 109.5 t ha⁻¹ [7]. Under this production system and other, more conventional systems [8, 9], organic or combined fertilization is required to enhance fig productivity, regardless of plant guiding, pruning, pest, disease and weed control, irrigation [10] and plant nutrition [3, 9, 11, 12]. The seasonal variation of macro- and micronutrients in high- and low-vigor fig tree orchards in California was studied [11], but no production values were offered. In contrast, a four-year field study concluded that the best fig yield and optimal macronutrients were achieved by applying 200 kg NH₄NO₃ ha⁻¹, 250 kg Ca(H₂PO₄)₂ ha⁻¹, 200 kg KCl ha⁻¹ and 1 t ha⁻¹ organic matter [13]. Another field experiment studied the effect of an intensive fertigation program with K and Ca plus N in 'Black Mission' and 'Sierra' fig trees [14]. This study suggested that fig plants treated with K and Ca had the greatest fruit size and marketable yields, but fruit quality, measured as total soluble solids concentration and titratable acidity, remained unchanged. To our best knowledge, no previous studies tested a full fertilization matrix of N, P, and K in fig trees, neither under field conditions nor under intensive culture, to determine the effects on leaf nutrient concentrations, fruit yield, and dry biomass of fig plants. Therefore, this research focused on evaluating the effect of a complete matrix of N, P, and K on the leaf concentration of macro- and micronutrients and dry biomass of 'Black Mission' fig plant organs under an intensive culture system and protected environment.

2. MATERIAL AND METHODS

2.1 Experimental site

The experiment was conducted from April to November 2016 at the Campo Experimental La Laguna, located in Matamoros, Coahuila, Mexico (25°31'56.8" LN y 103°14'29.9" LO). This research station belongs to the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias of Mexico. The experiment was set up under a macro tunnel equipped with a shade mesh with 50% sunlight attenuation.

2.2 Plant material and experimental design

Two-year-old fig plants (cv. 'Black Mission') previously propagated from stem cuttings were used. Plants were transplanted in pots (20 L volume) and sand substrate previously washed was used as growing media. There were three application rates each for N (0, 80, and 160 kg ha⁻¹), P (0, 40 and 80 kg ha⁻¹), and K (0, 80, and 160 kg ha⁻¹) arranged in a balanced factorial matrix of treatments 3³. The 27 mineral nutrient treatments were strictly randomized in each block and there were four replicates. The experimental unit comprised one plant per replicate (four replicates) per treatment.

2.3 Plant material and experimental design

The nutritive mineral solutions were designed from three mother nutrient solutions, with the following chemical fertilizers: solution 1: 4% Fe⁺³, 3% Mn⁺², 0.5% Cu⁺², 4% Zn⁺², 1.5% B⁺³ and 0.05% Mo⁺⁶; solution 2: 98 g L⁻¹ H₃PO₄; and solution 3: 132 g L⁻¹ (NH₄)₂SO₄, 236 g L⁻¹ Ca(NO₃)₂, 74.5 g L⁻¹ KCl, 65 g L⁻¹ CaSO₄, and 246.5 g L⁻¹ MgSO₄.

Irrigation was applied twice weekly based on Class-A pan evaporimeter readings. Starting in May, the fertigation program was carried out once weekly, splitting the nutrients through the growing season. Solution 1 was applied equally to all treatments; while solutions 2 and 3 were divided into three solutions for P and nine solutions for N and K. The electrical conductivity of the irrigation water used was 1.15 dS m⁻¹.

2.4 Biomass, leaf sampling, nutrient analysis

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After harvest in November, each plant was separated into roots, stems, leaves, and fruit and oven-dried at 60°C for a week to constant mass. The dry mass of all plant organs was added and expressed as total dry biomass.

To determine macro- and micronutrient concentrations, four mature leaves per experimental plant were taken to the local soil analysis lab. The leaves were washed individually with demineralized water twice, oven-dried at 65°C for a week to constant mass, and the dried samples were ground. N was analyzed by the calcination method (Flash 2000 organic element analyzer). P was determined by the colorimetric method with molybdate-vanadate. The K, Ca, Mg, Fe, Cu, Mn, Zn, and Na were determined by calcination in muffle and wet-digestion with 37% HCl in a Perkin Elmer AA-700 atomic absorption analyzer [15].

2.5 Nutrient use efficiency

The nutrient efficiency, in terms of agronomic efficiency (AE), was estimated as the additional economic yield per nutrient applied: $AE = \frac{(TBF (g) - TBNF (g))}{\text{Amount of fertilizer applied (g)}} = g g^{-1}$, where

TBF and TBNF are the total biomass of fertilized or non-fertilized plants, respectively [16, 17].

2.6 Statistical analysis

Data were analyzed in a randomized complete block model with three factors (N, P, and K) using the general linear model (GLM) procedure. Treatment means were grouped by the Fisher's test at $P = 0.05$. All calculations were performed in the statistical analysis system (SAS version 9.3; SAS Institute, Cary, NC, USA).

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3. RESULTS

3.1 Leaf nutrient concentrations

In addition to the significant influence of the main effects of N, P, and K over the foliar concentrations of almost all nutrients, the analysis found some significant interactions. For instance, the N x P x K and P x K interactions affected the foliar concentration of N, the N x K interaction affected the foliar concentrations of Ca and Mg, and the N x P interaction influenced all nutrient foliar concentrations except for Fe and Zn (Table 1). Therefore, the N x P interaction was the most important one to be explained.

Table 1. Summary of the analysis of variance of the NPK influence on the macro and micronutrient foliar concentrations of 'Black Mission' figs.

Source of variation	Macronutrients					Micronutrients			
	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
N	0.0001	0.0001	0.01	0.0001	0.0001	0.0001	0.38	0.0001	0.34
P	0.0002	0.0001	0.001	0.0001	0.0001	0.03	0.03	0.0001	0.08
K	0.58	0.66	0.01	0.01	0.05	0.12	0.26	0.80	0.35
N x P	0.03	0.0001	0.01	0.0001	0.0001	0.56	0.001	0.0002	0.37
N x K	0.95	0.38	0.24	0.001	0.05	0.58	0.90	0.27	0.33
P x K	0.0002	0.33	0.74	0.49	0.59	0.42	0.96	0.19	0.29
N x P x K	0.03	0.32	0.34	0.10	0.97	0.28	0.89	0.31	0.21
CV (%)	16.3	21.6	26.3	25.4	12.5	27.3	41.4	31.5	56.5

CV: is the coefficient of variation

At the end of the growing season, the leaf concentrations of N (Fig. 1A), P (Fig. 1B), and Mn (Fig. 2B) were greatest with N and P at 160 and 40 kg ha⁻¹, respectively. Applying > 40 kg ha⁻¹ P did not enhance leaf N, P, and Mn concentrations. In contrast, supplementary N and P at any level reduced the foliar concentrations of K, Ca, and Mg (Fig. 1C, 1D, and 1E, respectively). Foliar Cu was greatest with 80 kg ha⁻¹ N and 0 kg ha⁻¹ P, followed by 160 kg ha⁻¹ N and 80 kg ha⁻¹ P (Fig. 2A).

The main effect of N was to reduce leaf Fe concentrations. Mean values for Fe (least significant difference, LSD = 21.2 mg kg⁻¹) were 186.0, 172.1, or 137.5 with application of 0, 80, or 160 kg ha⁻¹ N, respectively. The greatest leaf Fe concentration was produced by 80 kg ha⁻¹ P. The mean values of leaf Fe with application of 0, 40 or 80 kg ha⁻¹ P were, 168.4, 149.5 or 177.8 mg kg⁻¹ (LSD = 21.2 mg kg⁻¹), respectively. Neither the interaction nor the main effect of N, P, and K altered leaf Zn concentration. Consequently, the interpretation of the interactions P x K and N x P x K on leaf N concentration and N x K on leaf Ca and Mg foliar concentrations were considered redundant. However, in the last interaction, supplementary K, at any rate, reduced leaf Ca and Mg concentrations (data not shown).

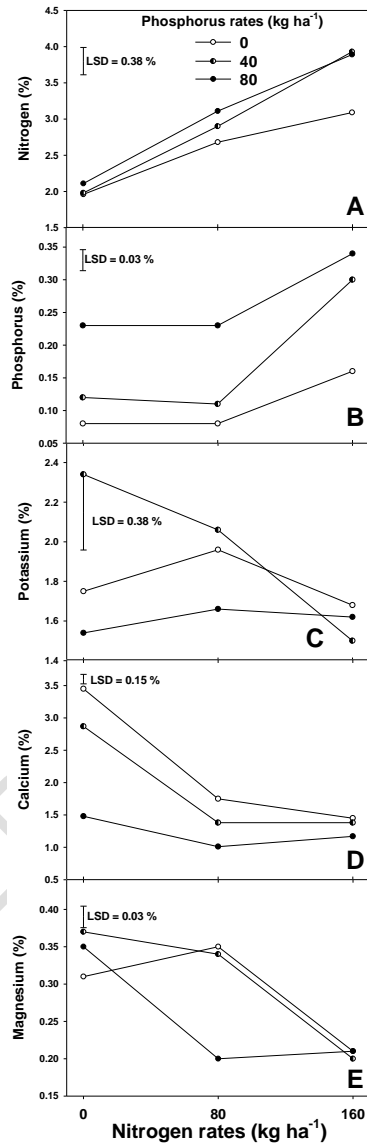


Fig. 1. (a) Effect of the interaction nitrogen x phosphorus (b) ?? (c) ?? on macronutrient concentrations (% dry mass) of 'Black Mission' fig leaves sampled after harvest in November. At each plot, the vertical bar is the Fisher's least significant difference (LSD) at $P = 0.05$.

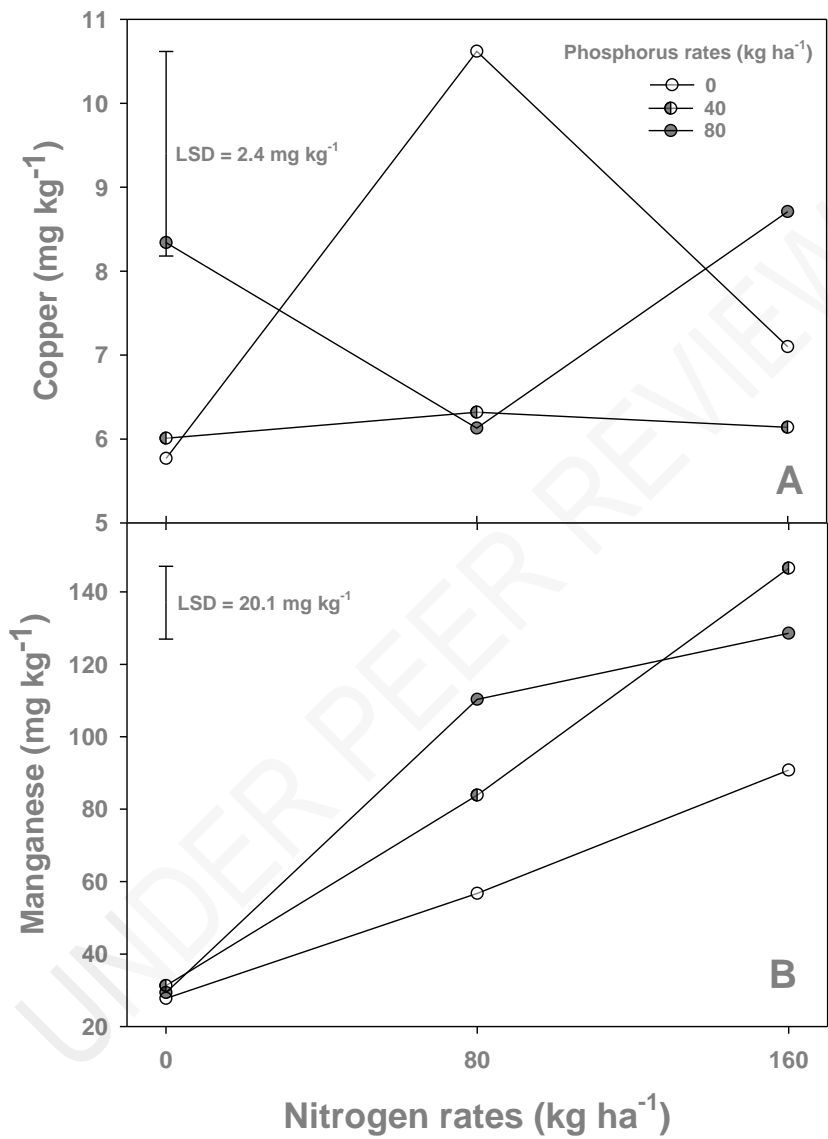


Fig. 2. Effect of the interaction nitrogen x phosphorus on copper (A) and manganese (B) concentrations (% dry mass) of 'Black Mission' fig leaves sampled after harvest in November. At each plot, the vertical bar is the Fisher's least significant difference (LSD) at $P = 0.05$.

3.2 Association among leaf nutrient concentrations

Leaf N concentration correlated moderately and positively (synergism) with leaf P and Mn concentrations, but correlated from low to moderately and negatively (antagonism) with leaf Fe, Ca, and Mg concentrations. Similar antagonism was found between leaf P concentration and leaf Fe, K, Ca, and Mg concentrations, but a weak and significant synergism with leaf Cu concentration occurred. Leaf K concentration had a low synergism with leaf Mg and Cu concentrations, while leaf Ca concentration had low synergism and antagonism with leaf Mg, Cu and Mn concentrations, respectively. Leaf Fe concentration correlated weakly and negatively with leaf Mn concentration, but there was weak synergism and moderate antagonism between leaf Mg concentration and leaf Fe and Mn concentrations, respectively (Table 2).

Table 2. Pearson correlation coefficients among leaf nutrient concentrations of fig plants cv. 'Black Mission'.

	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
N	1								
P	0.59***	1							
K	-0.09	-0.35***	1						
Ca	-0.55***	-0.53***	0.18	1					
Mg	-0.57***	-0.62***	0.47***	0.49***	1				
Fe	-0.34***	-0.24**	-0.04	0.20	0.30***	1			
Cu	0.15	0.01	0.32***	-0.25**	0.14	0.14	1		
Mn	0.70***	0.56***	-0.18	-0.47***	-0.63***	-0.24**	-0.05	1	
Zn	0.02	-0.00	-0.01	-0.12	-0.04	0.04	0.03	0.07	1

Correlation coefficients were significant at ** = 0.01 or *** = 0.0001; otherwise, non-significant

3.3 Dry biomass components

The main effect of N or K altered root dry mass, while N or P affected fruit dry mass. The other fig plant organs, including total dry biomass, were modified ($P < 0.01$) by the interaction of N x P or N x K on shoot dry mass only (Table 3).

Table 3. Summary of the analysis of variance of the NPK influence on the dry mass of fig plants organs cv. 'Black Mission'.

Source of variation	Dry mass (g)				
	Root	Shoot	Leaves	Fruit	Total biomass
N	0.0001	0.0001	0.0001	0.006	0.0001
P	0.20	0.0001	0.0001	0.005	0.0001
K	0.02	0.0004	0.15	0.32	0.01
N x P	0.42	0.0001	0.01	0.08	0.004
N x K	0.46	0.02	0.15	0.40	0.23
P x K	0.19	0.90	0.33	0.53	0.18
N x P x K	0.26	0.06	0.59	0.68	0.17
CV (%)	28.6	38.7	41.9	78.8	27.7

CV_i is the coefficient of variation

Root dry biomass improved from 0 to 80 kg ha⁻¹ N, but 160 kg ha⁻¹ N tended to reduce the dry mass accumulation of this plant organ (Fig. 3A). In contrast, the addition of K, at any rate, consistently reduced root dry mass (Fig. 3B)

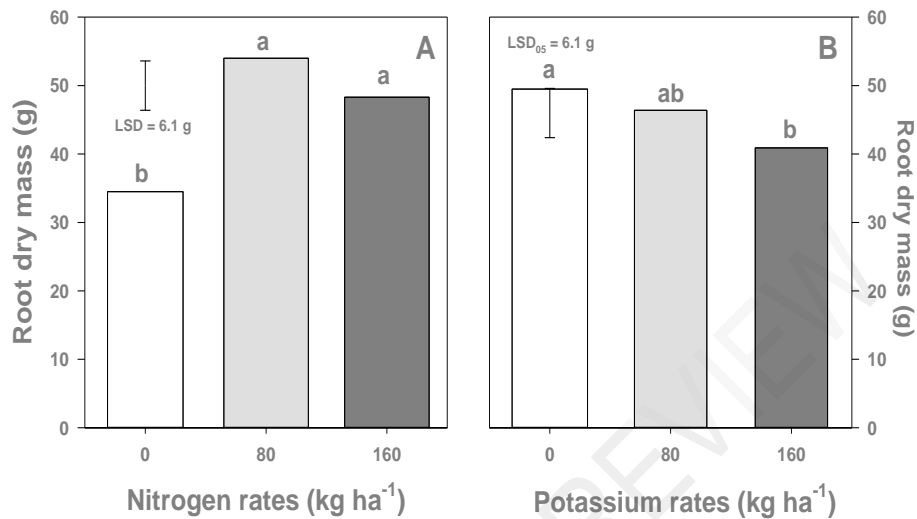


Fig. 3. Influence of the main effect of nitrogen (A) and potassium (B) rates on root dry mass of 'Black Mission' fig plants. At each plot, the vertical bar is the Fisher's least significant difference (LSD) at $P = 0.05$.

The interaction N x P, at 80 kg ha⁻¹ N and 40 kg ha⁻¹ P, produced the most shoot dry mass (Fig. 4A); in contrast, the highest shoot dry mass with the interaction N x K occurred at 80 kg ha⁻¹ N and 0 kg ha⁻¹ K (Fig. 4B). Similar leaf dry mass was observed with the interaction N x P, either at 80 kg ha⁻¹ N and 40 kg ha⁻¹ P or at 80 kg ha⁻¹ N and 80 kg ha⁻¹ P (Fig. 4C). Increasing N and P beyond these rates limited dry mass accumulation in shoots and leaves; while adding K harmed shoot dry mass accumulation at any rate (Fig 4).

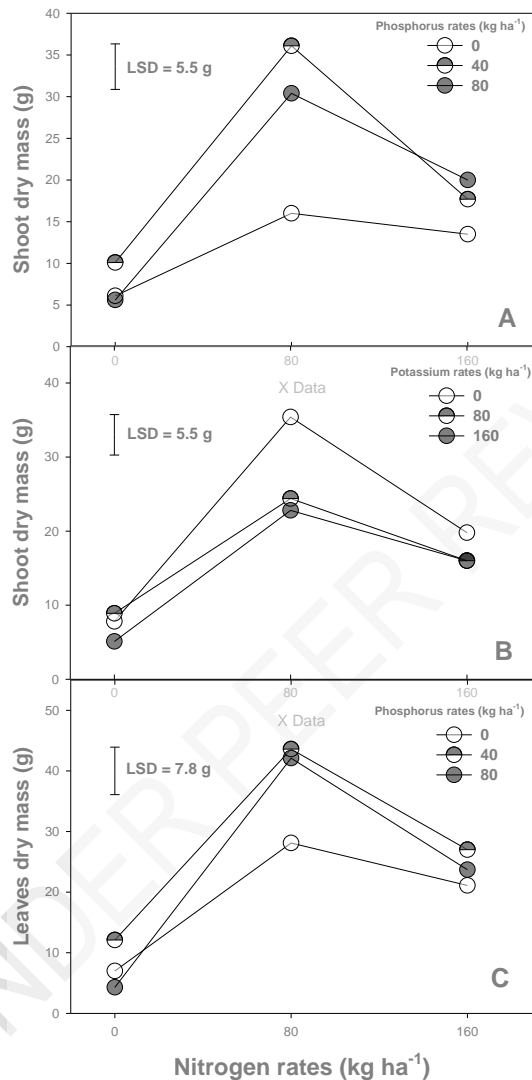


Fig. 4. Effect of the interaction nitrogen x phosphorus on the dry mass of shoots (A) and leaves (C) and effect of the interaction nitrogen x potassium on shoot dry mass (B) of 'Black Mission' fig plants. At each plot, the vertical bar is the Fisher's least significant difference (LSD) at $P = 0.05$.

Fruit dry biomass increased from 0 to 80 kg ha⁻¹ N, while 160 kg ha⁻¹ N tended to reduce dry mass accumulation in this plant organ (Fig. 5A). In contrast, fruit dry mass was greatest at 160 kg ha⁻¹ P. However, total dry mass responded positively to the interaction N x P at the rates of 80 kg ha⁻¹ N and 40 kg ha⁻¹ P and of 80 kg ha⁻¹ N and 80 kg ha⁻¹ P. Total dry mass accumulation was reduced at the highest application rates of N and P (Fig. 5C).

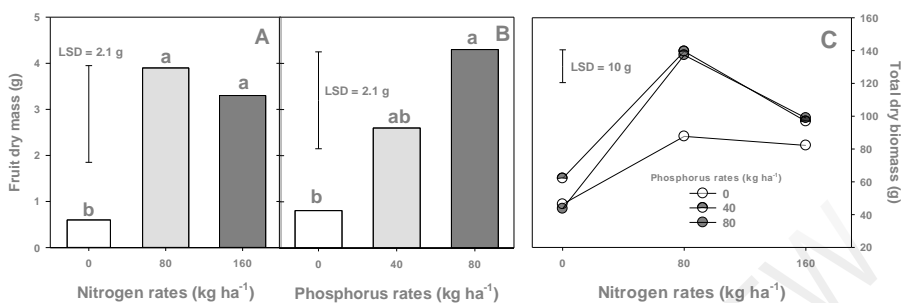


Fig. 5. Main effect of nitrogen (A) and phosphorus (B) rates on fruit dry mass and effect of the interaction nitrogen x phosphorus (C) on total dry biomass of 'Black Mission' fig plants. At each plot, the vertical bar is the Fisher's least significant difference (LSD) at $P = 0.05$.

3.3 Agronomic use efficiency of mineral nutrients

Analysis of the agronomic efficiency of nutrient applications suggested that applying K, at any rate, reduced total biomass, as did applying more than 80 kg N ha⁻¹ and 40 kg P ha⁻¹ (Table 4).

Table 4. Agronomic efficiency in the use of N, P, and K in 'Black Mission' fig plants.

NPK rates (kg ha ⁻¹)	Agronomic efficiency in the use of nutrients (g g ⁻¹)
Nitrogen	
0	—
80	159.6a*
160	40.3b
Significance ($p > F$)	0.0001
Least significant difference	32.0
Phosphorus	
0	—
40	196.8a
80	81.8b
Significance ($p > F$)	0.002
Least significant difference	71.4
Potassium	
0	—
80	71.5a
160	34.3b
Significance ($P > F$)	0.03
Least significant difference	33.6

*Mean separations within the column per nutrient was by Fisher's test ($P = 0.05$). Mean values followed by different lower-case letter were significantly different

4. DISCUSSION

In crop plants, an interaction between nutrients takes place when application of one nutrient stimulates or suppresses absorption and use of other nutrients [18]. Here, when N and P were supplied at any rate, both mineral fertilizers increased leaf N, P, and Mn

concentrations, but decreased leaf K, Mg, Ca, Cu, and Fe concentrations (Fig. 1 and 2, Table 2). This last behavior was reinforced by correlating the N and P rates with leaf K, Mg, Ca, Fe and Cu concentrations, which for N were: -0.21 ($P = 0.03$), -0.70 ($P = 0.0001$), -0.56 ($P = 0.0001$), -0.39 ($P = 0.0001$), and 0.08 ($P = 0.42$), respectively. The values for P rates with K, Mg, Ca, and Cu were: -0.14 ($P = 0.14$), -0.20 ($P = 0.03$), -0.44 ($P = 0.0001$), and -0.01 ($P = 0.89$), respectively. Nevertheless, at 80 kg ha^{-1} N and 40 kg ha^{-1} P, the synergisms and antagonisms allowed similar leaf nutrient concentrations to those reported in leaves postharvest collected (October) from high-vigor mature fig trees [11]. Our mean (\pm SD) leaf nutrient concentration values (percentage of dry weight) for N, P, K, Ca, and Mg were 2.9 ± 0.3 , 0.11 ± 0.01 , 2.1 ± 0.4 , 1.4 ± 0.7 , and $0.34 \pm 0.03\%$, respectively; and concentrations of Fe, Cu, Mn, and Zn were 166 ± 49 , 6.3 ± 1.7 , 83 ± 21 , and $22 \pm 4 \text{ mg kg}^{-1}$, respectively. These leaf nutrient concentration values are consistent with previously reported in vigorous and healthy fig trees [19, 20]. The discrepancies in some macro- and micronutrient concentrations compared to those reported previously could be attributed to the plant vigor, fertilization program (fig plants received N only), and genetic material [11, 21].

The interaction of 80 kg ha^{-1} N and 40 kg ha^{-1} P enhanced the dry mass of shoots (Fig. 4A), leaves (Fig. 4C), and total biomass (Fig. 5C); while the main effect of 80 kg ha^{-1} N also improved dry mass of roots (Fig. 3A) and the main effects of 80 kg ha^{-1} N and 40 kg ha^{-1} P enhanced fruit dry mass (Fig. 5B). Both mineral nutrients are important components in photosynthesis. Nitrogen is part of many carbon compounds [22] and phosphorus is used in energy storage and transfer [23]. Our data suggest that this level of interaction can maintain leaf Mg concentrations adequately, as the main component of the chlorophyll molecule, and favor of greater dry mass in all fig plant organs. Higher rates of N and P negatively affected leaf concentrations of some nutrients (antagonism) (Fig. 1 and 2, Table 2 and 4) [24] and dry mass of all plant organs (Fig. 3 - 5) due to superfluous consumption (phytotoxic effect) of these nutrients during fig plant growth [18].

In leaf tissue, K^+ is necessary for osmoregulation and cell turgor maintenance [25]. However, leaf Ca and Mg concentrations were reduced when K was supplied at any level (data not shown). Therefore, even when non-significant, both Ca and Mg correlated negatively with K^+ (Ca, $r = -0.14$ and Mg, $r = -0.10$). This antagonism was reflected in both root dry mass (Fig. 3B) and shoot dry mass (Fig. 4B). This was possible because Ca and Mg are basic nutrients in plant cell growth (middle lamella and wall of cells) and photosynthesis (chlorophyll molecule), respectively [22]. This suggests that K^+ contained in the irrigation water (electrical conductivity = 1.15 dS m^{-1}) was sufficient enough to meet the fig plants' requirements and additional supplementation induced a phytotoxic effect in these fig plants [16, 17]. Thus, K addition to our growing media had an opposite effect to those in previous reports where K was supplied to fig plants [13, 14, 26, 27]. It has been argued that a leaf K concentration between one and three % is adequate for good plant performance during the growing season [22, 28, 29]. However, specifically in 'Calimyrna' fig plants, the postharvest leaf K concentration (October) was between $0.25 (\pm 0.2\%)$ and $0.7 (\pm 0.3\%)$ in low-vigor and high-vigor plants, respectively [11]. Leaf K concentrations in 'Sierra' plants in September, non- or fertigated with $12.6 \text{ units of K ha}^{-1}$, ranged between 1.66% and 1.52% , respectively, and the corresponding values for 'Black Mission' leaf K concentrations were 1.08% and 1.42% , respectively [14]. Here, in the interaction N x P, leaf K and Ca concentrations (mean \pm SD) were $2.1 \pm 0.4\%$ and $1.4 \pm 0.7\%$, respectively. These values were within the normal range of leaf K ($2.1 \pm 0.5 \%$) and Ca ($1.9 \pm 0.4\%$) concentrations reported for mature fig plants sampled in October [19] and to leaf K and Ca concentrations reported in other deciduous fruit trees sampled during late July and August [30].

According to [31], if the nitrogen levels are high, an imbalance between the foliar and the root area can occur. If this occurs, the larger leaf area causes an increase in transpiration that is not corresponded to an increase in water absorption [32], therefore, the plant can enter into water stress [33], although it has good water availability and fruit blossom end rot

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may appear [34, 35]. Soil analysis and foliar analysis are the instruments used in monitoring productivity performance in relation to the control of phosphorus levels and is key to deciding the use of phosphate fertilizers in the field. Also, when potassium is at adequate levels, it is recommended to maintain the level of the nutrient in the soil by applying only what is extracted by the crop.

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5. CONCLUSION

The N x P interaction at 80 kg ha⁻¹ N and 40 kg ha⁻¹ P produced the most total dry biomass and its components. At higher applications of N and P, a phytotoxic effect ~~occurred~~ occurred, and total dry biomass was reduced, which was corroborated by the analysis of the agronomic efficiency of N and P applications. The rate of 80 kg ha⁻¹ N and 40 kg ha⁻¹ P induced both synergism and antagonism among leaf nutrient concentrations resulting in final postharvest leaf nutrient concentrations of 2.9% N, 0.11% P, 2.1% K, 1.4% Ca, 0.34% Mg, 166 mg kg⁻¹ Fe, 6.3 mg kg⁻¹ Cu, 83 mg kg⁻¹ Mn, and 22 mg kg⁻¹ Zn. The addition of supplemental K deserves further study.

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CONSENT (WHERE EVER APPLICABLE)

Not applicable for this manuscript.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable for this manuscript.

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