FOLIAR NUTRIENT CONCENTRATION AND MICRONUTRIENT UPTAKE IN THREE PINEAPPLE VARIETIES ESTABLISHED AT DIFFERENT PLANTING DENSITIES †

[CONCENTRACIÓN FOLIAR DE NUTRIENTES Y ABSORCIÓN DE MICRONUTRIENTES EN TRES VARIEDADES DE PIÑA ESTABLECIDAS A DIFERENTES DENSIDADES DE PLANTACIÓN]

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SUMMARY

Background. The amount of micronutrients required by pineapple changes according to different factors, including cultivar and planting density. Knowing the micronutrient requirement in quantity and the appropriate phenological stage will allow the development of an adequate fertilization program. **Objectives.** (1) to determine the content of Cu, Fe, Mn, and Zn during the development of three pineapple varieties, at three planting densities; (2) to determine the effect of planting densities on the foliar concentration of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn during the vegetative stage of the plants; (3) to determine the effect of foliar concentrations on the total micronutrient content at harvest. **Methodology.** The varieties 'Smooth cayenne', 'Champaka' and 'MD-2' were evaluated at densities of 30 000, 45 000, and 60 000 plants ha^{-1}. The experimental design was a randomized block. The treatments were arranged in a split-plot design, with four replications. The Cu, Fe, Mn, and Zn contents were determined in eight samplings, and the concentrations of N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn were measured in the first five samplings. **Results.** Higher Cu, Fe, Mn, and Zn content per plant was detected at 30,000 plants ha-1, but higher extraction per hectare was observed at 60,000 plants ha^{-1}. Similar Fe, Mn, and Zn contents were detected among varieties. No defined behavior of nutrient concentration was detected in leaf D. In the three varieties, a high Pearson correlation ($r \geq 0.5$) was detected between concentrations and total micronutrient content at 3.6, 4.6, 6.3, 8.6, and 10.1 months of age. **Conclusions.** The amount of Fe, Mn, Cu, and Zn extracted increases as planting density increases, reaching maximum values of 12.7, 6.2, 1.2, and 0.6 kg ha^{-1}. The Fe extraction of the cv. 'MD-2' is 30% lower than that of the 'Smooth cayenne' and 'Champaka'. A high correlation between leaf nutrient concentration and total Fe, Mn, Cu and Zn content was detected only 18% of the time during the flower induction stage (at 10.1 months of age). A high correlation was detected 82% of the time in samples taken between 4 and 9 months after planting. Leaf analysis was found to be most effective for predicting Fe and Zn behavior, but less effective for Mn and Cu behavior in leaf D.

Key words: *Ananas comosus*; 'Champaka'; Copper; Iron; Leaf D; Manganese; 'MD-2'; 'Smooth cayenne'; Zinc.

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RESUMEN

Antecedentes. La cantidad de micronutrientes requeridos por la piña cambia en función de diferentes factores, entre ellos se encuentra, el cultivar y la densidad de plantación. Conocer el requerimiento nutrimental en cantidad y etapa fenológica oportuna, permitirá desarrollar un adecuado programa de fertilización. **Objetivo.** (1) determinar el contenido del Cu, Fe, Mn y Zn durante el desarrollo de tres variedades de piñas, en tres densidades de plantación; (2) determinar el efecto de las densidades de plantación en la concentración foliar de N, P, K, Ca, Mg, Cu, Fe, Mn y Zn durante la etapa vegetativa de las plantas; (3) determinar el efecto de las concentraciones foliares sobre el contenido total micronutrientes al momento de la cosecha. **Metodología.** Se evaluaron las variedades 'Smooth cayenne', 'Chapaka' y 'MD-2', en las densidades de 30 000, 45 000 y 60 000 plantas ha^{-1}. El diseño experimental fue bloques al azar. El arreglo de tratamientos fue en parcelas divididas, con cuatro repeticiones. Se determinó el contenido de Cu, Fe, Mn y Zn en ocho muestreos y la concentración de N, P, K, Ca, Mg, Fe, Mn, Cu y Zn en los primeros cinco muestreos. **Resultados.** Mayor contenido Cu, Fe, Mn y Zn por planta se detectó con 30 000 plantas ha^{-1}, pero una mayor extracción por hectárea con 60 000 plantas ha^{-1}. Similar contenido de Fe, Mn y Zn se detectó entre variedades. No se detectó un comportamiento definido de la concentración de nutrientes en la hoja D. En las tres variedades se detectó una alta correlación de Pearson ($r \geq 0.5$) entre las concentraciones y el contenido total de micronutrientes a los 0.6, 4.6, 6.3, 8.6 y 10.1 meses de edad. **Implicaciones.** Se identificó los cambios que pueden ocurrir en el requerimiento de Fe, Mn, Cu y Zn en función del cultivar de piña, la densidad de plantación y la etapa fenológica de la planta. Esta información será útil para productores, técnicos agrícolas e investigadores de México y del mundo, para generar programas de fertilización o para nuevas investigaciones. **Conclusión.** La cantidad de Fe, Mn, Cu y Zn extraído aumenta medida que aumenta la densidad de plantación, alcanzando valores máximos de 12.7, 6.2, 1.2 y 0.6 kg ha^{-1}. La extracción de Fe en el cv. 'MD-2' es 30% menor que el de 'Cayena Lisa' y 'Champaka'. Una alta correlación entre la concentración foliar de nutrientes y el contenido total de Fe, Mn, Cu y Zn, solo detectó en 18% de las ocasiones durante la etapa de inducción floral (a los 10.1 meses de edad). El 82% de ocasiones una alta correlación se detectó en muestreos realizados entre los 4 y 9 meses de edad de la planta. Se encontró que el análisis foliar es más efectivo para predecir el comportamiento del Fe y Zn, pero menos efectivo para el comportamiento de Mn y Cu en la hoja D.

Palabras claves: *Ananas comosus*; 'Champaka'; Cobre; Fierro; Hoja D; Manganeso; 'MD-2'; 'Cayena Lisa'; Zinc.

INTRODUCTION

Pineapple is a highly valued tropical fruit worldwide. In 2023, a world production of 29.3 million tons was reported (Shahbandeh, 2024). Mexico stands out as the ninth-largest producer, producing 1.272 million tons of fresh fruit in 2022 (SIAP, 2023). Of the 32 states in the Mexican Republic, 14 states produce pineapples, with Veracruz standing out as the largest producer, accounting for 82% of the area planted with pineapple (SIAP, 2023).

Pineapple requires high amounts of nutrients, which should preferably be supplied through site-specific fertilization (Khuong *et al.*, 2024). The most required nutrients are nitrogen (N) and potassium (K). In Mexico, it has been found that, depending on planting density, 377 - 609 and 449 - 875 kg ha^{-1} can be extracted in one production cycle, respectively (Rebolledo-Martínez *et al.*, 2023). The other most extracted nutrients are calcium (Ca), phosphorus (P), magnesium (Mg) and sulfur (S), in all cases, the amount extracted by plants is greater than 50 kg ha^{-1} (Silva *et al.*, 2009; Souza *et al.*, 2019; Rebolledo-Martínez *et al.*, 2023). For this reason, N, P, K, Ca, and Mg are mainly nutrients considered in fertilization programs (Rebolledo Martínez *et al.*, 2016; Uriza-Ávila *et al.*, 2018).

Most research on pineapple nutrition has focused on macronutrients, with a minimal amount of attention given to micronutrients. This scarce attention to micronutrients is reflected in the little importance given by agricultural technicians and growers to the application of copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). In Mexico, the application of micronutrients is recommended during the vegetative period, from planting to the time of flower induction treatment (Rebolledo Martínez *et al.*, 2016; Uriza-Ávila *et al.*, 2018). However, it is uncertain whether such recommendations adequately meet the needs of plants. Most reports on micronutrients are outdated. (Hiroce *et al.*, 1977) reported that pineapple 'Smooth cayenne' at a density of 50,000 plants ha^{-1} extracted 0.40, 0.19, 5.09, and 2.25 kg ha^{-1} of Zn, Cu, Fe, and Mn, respectively. It was reported that 'Perola' pineapple, established at 50,000 plants ha^{-1} extracted 0.337, 0.169, 4.020, and 7.308 kg ha^{-1} of Zn, Cu, Fe and Mn, whereas

'Smooth cayenne' extracted 0.225, 0.197, 4.793, and 6.351 kg ha^{-1}, respectively (Paula *et al.*, 1985). Another study mentions that, in 30 tons of pineapple residues, 8.1, 5.4, 0.3 and 0.9 kg ha^{-1} of Mn, Fe, Cu, and Zn were extracted (Py *et al.*, 1987). On the other hand, Hanafi *et al.* (2009) report that the varieties 'Gandul', 'N-36', 'Moris', 'Josapine', and 'Sarawak' extract of about 0.15 - 12.7 and 3.00 - 17.04 g plant^{-1} of Cu and Fe, respectively.

Pineapples produced in Mexico have different destinations, approximately 5% of the fresh export market, about 20% for industry, and the rest is consumed fresh in the national market (Uriza-Ávila *et al.*, 2018). Different markets demand different fruit weights, so plantations must be established at different planting densities to obtain the sizes required by consumers (Rebolledo Martínez *et al.*, 2006; Cardoso *et al.*, 2013; Souza *et al.*, 2019). Another characteristic of Mexico is that two varieties of pineapple currently dominate the market. Official statistics indicate that 67% and 30% of the national area is planted with 'Smooth cayenne' and 'MD-2' (SIAP, 2023). However, by 2024, according to observations of researchers focused on pineapple crop and data from producers, about 80% of the planted area corresponds to 'MD-2', 15% to 'Smooth cayenne' and the remaining 5% to other varieties, such as 'Champaka', 'Cabezona de Tabasco', 'Coitia de Chiapas', 'Criolla de Guerrero', 'Crioya de Nayarit', among others. According to Uriza-Ávila *et al.* (2018), a promising pineapple genotype in Mexico is 'Champaka', which is expected to expand in the future. Both planting density and variety type influence plant development and fruit production (Hanafi *et al.*, 2009; Neri *et al.*, 2021). These differences will also be reflected in the nutritional aspect during plant development and in the amount of nutrients they will extract at harvest (Sampaio *et al.*, 2011; Cardoso *et al.*, 2013; Souza *et al.*, 2019; Trejo *et al.*, 2020).

Agronomic practices and various biotic or abiotic factors can affect the soil nutrient pool. Deficient, adequate, or excessive levels of nutrients in the soil will be reflected in pineapple plants. In some cases, the nutrient status of a plant can be observed through visual symptoms, but in other cases, it can only be determined through chemical analysis. Leaf analysis is the most commonly used diagnostic tool to monitor the nutritional status of pineapple during its growth cycle. This analysis is specifically performed in the group of leaves denominated as leaf D. The reason is that it can be easily identified and is the youngest physiologically active leaf (Vázquez-Jiménez and Bartholomew, 2018). The concentration of nutrients in leaf 'D' at the time of floral induction has a high correlation with fruit weight and the total weight of the plant, so this phenological stage is the most used for leaf analysis (Vilela *et al.*, 2015). Optimal foliar concentration ranges for certain micronutrients and specific pineapple varieties have already been identified in various regions of the world (Vázquez-Jiménez and Bartholomew, 2018). However, in Mexico, this information is still insufficient, and it is also not known how foliar nutrient concentration affects total micronutrient content at harvest. Therefore, three objectives were considered in this study: (1) to determine the content of Cu, Fe, Mn and Zn during the development of three pineapple varieties established at three planting densities; (2) to determine the foliar concentration of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn during the vegetative stage of the plants as a function of the three planting densities and; (3) to know the effect of foliar concentrations on the total content of Cu, Fe, Mn, and Zn at the time of fruit harvest of the pineapple varieties. Two hypotheses were initially proposed: (1) the total micronutrient content changes according to the variety and planting density; (2) the foliar concentration of nutrients at flower induction will have a high correlation with the total micronutrient extraction at harvest.

MATERIALS AND METHODS

Study area

An experiment was stablished in the Papaloapan Basin, within the facilities of the 'Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias' (INIFAP). The 'Papaloapan' Experimental Station of the INIFAP is in a region with an Aw_0 climate (García, 2004). The soil used was a dystric cambisol type, poor in organic matter and nutrients: 4.8 pH; electrical conductivity of 0.043 dS m^{-1}; 0.92 and 0.053 % of organic matter and N, respectively; 3 mg kg^{-1} P; 0.6, 0.74, and 0.08 cmol$^{(+)}$ kg^{-1} K, Ca, and Mg; 0.34, 50, 1.23, 0.90 and 16 mg kg^{-1} of Na, Fe, Cu, Zn and Mn, respectively. Temperature and annual precipitation were recorded during the experiment (Figure 1).

Experimental design and treatments

Pineapple shoots 'MD-2', 'Smooth Cayenne' and 'Champaka' weighing 400 to 500 g were selected from the gene bank of Papaloapan Experimental Station. A split-plots treatment arrangement with a randomized block design and four repetitions was used. Planting density (30,000, 45,000, and 60,000 plants ha-1) was considered as a large plot, and the small plot was the pineapple variety. The experimental unit was 120 plants. The experimental unit consisted of three 9 m long planting beds (distance of 1.25 m from center to center of the beds) with two rows per bed spaced 45 cm.

Pineapple establishment and agronomic management

The field experiment was carried out without an irrigation application. Soil moisture depended only on rainfall. The planting was done in December 1997. The fertilization rate changed according to planting density. For 60000 plant ha^{-1} 12-8-12-4 was applied, for 45000 plant ha^{-1} the dose was 14-8-14-4 and for 30000 plant ha^{-1} the dose was 17-8-17-4 g plant^{-1} of N-P_2O_5-K_2O-Mg, respectively. For the three planting densities, 75% of the fertilizer dose was applied solidly, divided into three applications, at 229, 278 and 407 days after planting (DAP). The fertilizers used were urea (46% N), potassium sulfate (50% K_2O), potassium chloride (60% K_2O), diammonium phosphate (18-46% of N and P_2O_5) and magnesium sulfate (16% MgO). The remaining 25% of the dose was applied in liquid form, divided into five applications (at 464, 508, 537, 623 and 661 DAP). For each 200 L of water, 3, 3, 2 and 1 kg of urea, potassium chloride, diammonium phosphate and magnesium sulfate were applied, respectively. Each plant received 70 ml of the solution. Micronutrients were applied in liquid form, for 60000 plants ha^{-1} 3.0-2.8-2.18-1.2-2.4 was applied, for 45000 plants ha^{-1} 2.2-2.1-1.6-0.9-1.8 was applied and for 30000 plants ha^{-1} 1.5-1.4-1.1-0.6-1.2 kg ha^{-1} of Fe, Zn, B, Mn, and Cu, respectively. Weed control and pest control (symphylids, Mealybugs and nematodes) were carried out based on the recommendations of Rebolledo Martínez *et al.* (1998) using the recommended agrochemicals.

The flower induction treatment was carried out with calcium carbide dissolved in water at 2%. Three applications were made, at 304, 307 and 310 DAP. At each date, 60 mL of the solution was applied to each plant. The application was made between 18:00 and 22:00 hours. The fruits were harvested at 551 DAP, when the fruits presented an external ripening of 50% (Soler, 1990).

Leaf micronutrient concentration and nutrient uptake in pineapple plants

Eight plant samplings were made during the entire production cycle at 107, 153, 202, 278, 321, 441, 506 and 551 DAP. On each sampling date, one fully competent plant was obtained from each experimental unit and segmented into roots, stems, leaves (Only green leaves), peduncles, and fruits. On the other hand, from the first sampling until the moment of flower induction (107, 153, 202, 278 and 321 DAP), from each experimental unit, from a plant with complete competition, leaf D was collected to determine the nutrient concentration. The total fresh weight of each plant organ was recorded. Representative samples were obtained from each organ and dried in a forced air oven at 70 °C until constant weight was reached.

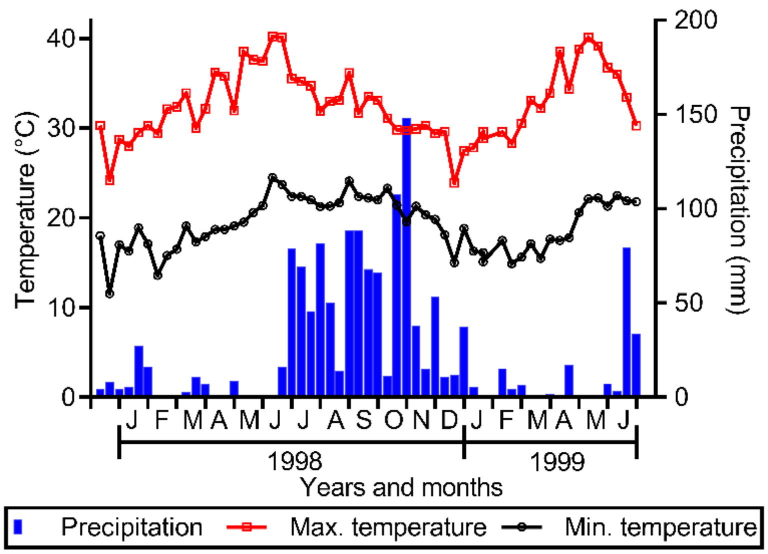

Figure 1. Behavior of temperature (maximum and minimum) and precipitation during development of three pineapple cultivars in Isla, Veracruz, Mexico from January (J) to June (J).

Dry matter was determined for each sample by gravimetry. The dry matter was subjected to analysis to determine the concentration of N by the micro Kjeldahl method (Bremner, 1965), P by the colorimetric method (Olsen *et al.* 1954), K by the atomic emission spectroscopy (Chapman *et al.*, 1973), and Ca, Mg, Cu, Fe, Mn, and Zn by atomic absorption spectroscopy (Bradfield and Spincer, 1965).

On each sampling date, the content of nutrients (Cn) in each plant organ was determined using the equation $Cn = nutrient\ concentration \times dry\ matter\ weight$. Likewise, the nutrient content per plant (Cnp) was calculated using the equation: $C_{np} = C_{roots} + C_{stem} + C_{leaves} + C_{peduncle} + C_{fruit}$ (Maia *et al.* 2016).

Data analysis

A regression analysis ($p \leq 0.05$) was performed to determine the behavior of Cu, Fe, Mn and Zn content in the plant and the concentration of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn in the leaf D over time, as a function of plant density. In all cases, the models with the highest coefficient of determination (R^2) were selected. A Pearson correlation analysis ($p \leq 0.05$) was performed to detect the relationship between the foliar concentration of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn during the vegetative growth stage and the content of Cu, Fe, Mn, and Zn in the total pineapple plant at the time of fruit harvest.

RESULTS

Copper, iron, manganese, and zinc content

The highest Cu, Fe, Mn, and Zn content in the plant was detected at 30 000 plants ha^{-1}, however, in the content per hectare, from 441 DAP, the highest values were detected at 60 000 plants ha^{-1} (Figure 2 a, b, c, d, e, f, g).

At harvest, Cu content per plant and per hectare was lower in 'MD-2' compared to ‘Champaka’ and ‘Smooth cayenne’ (Figure 3 a, d). As for Fe content, some differences were detected between 278 and 441 DAP, however, at harvest these differences between varieties were minimal (Figure 3 b, e). In the case of Mn and Zn, the contents per plant and per hectare were similar among varieties throughout the pineapple cycle (Figure 3, c, d, f, g). Of the total micronutrients absorbed by the three pineapple varieties, according to the regression models, at the time of flower induction (310 DAP), the plants had already absorbed 59, 70, 79 and 64% of Cu, Fe, Mn and Zn, respectively. At the end of flowering (441 DAP), plants had already taken up 81, 90, 97 and 87% of Cu, Fe, Mn, and Zn, respectively. The average of the three pineapple varieties indicates that 100% of the micronutrients were absorbed at harvest (551 DAP).

Leaf nutrient concentration

As for the concentration of nutrients in leaf D, no defined behavior was detected. Of the three planting densities, in at least one sampling, the density with 30 000 plants ha^{-1}, reached a higher concentration of N, P, K and Mg compared to the other densities (Figure 4 a, b, c, e). On the other hand, in at least one sampling, a higher concentration of Cu and Zn was detected with the density of 60 000 plants ha^{-1} compared to the other planting densities (Figure 4 f, i). At the flower induction stage, a higher concentration of Fe was detected at the density of 45 000 plants ha^{-1} compared to the other planting densities (Figure 4 g).

Correlation between foliar nutrient concentration and copper, iron, manganese and zinc content in the plant

For 'Smooth cayenne' pineapple, correlations with a "r" greater than 0.5 (high correlation) between leaf N concentration and Fe and Zn content were detected in three samplings and correlated with Cu and Mn content in one sampling; in all cases, the correlation was negative (Table 1). Leaf P concentration was negatively correlated with Fe content in four samples and with Cu, Mn, and Zn contents in one sample (Table 1). Leaf K concentration was correlated with Fe content in three samplings (two positive correlations and one negative correlation), with Zn content in four samplings (two negative correlations and two positive correlations) and was positively correlated with Cu content in one sampling (Table 1). Leaf Ca concentration was correlated with Fe content in three samplings (one positive correlation and two negative correlations), two negative correlations with Mn content and one positive correlation with Zn content (Table 1). Leaf Mg concentration was negatively correlated with Cu content in three samplings, was correlated with Mn and Zn content in two samplings (one positive correlation and one negative correlation) and was positively correlated with Fe content in one sampling (Table 1). Leaf Cu concentration was correlated with Fe and Zn content in four samples (two positive correlations and two negative correlations) and was positively

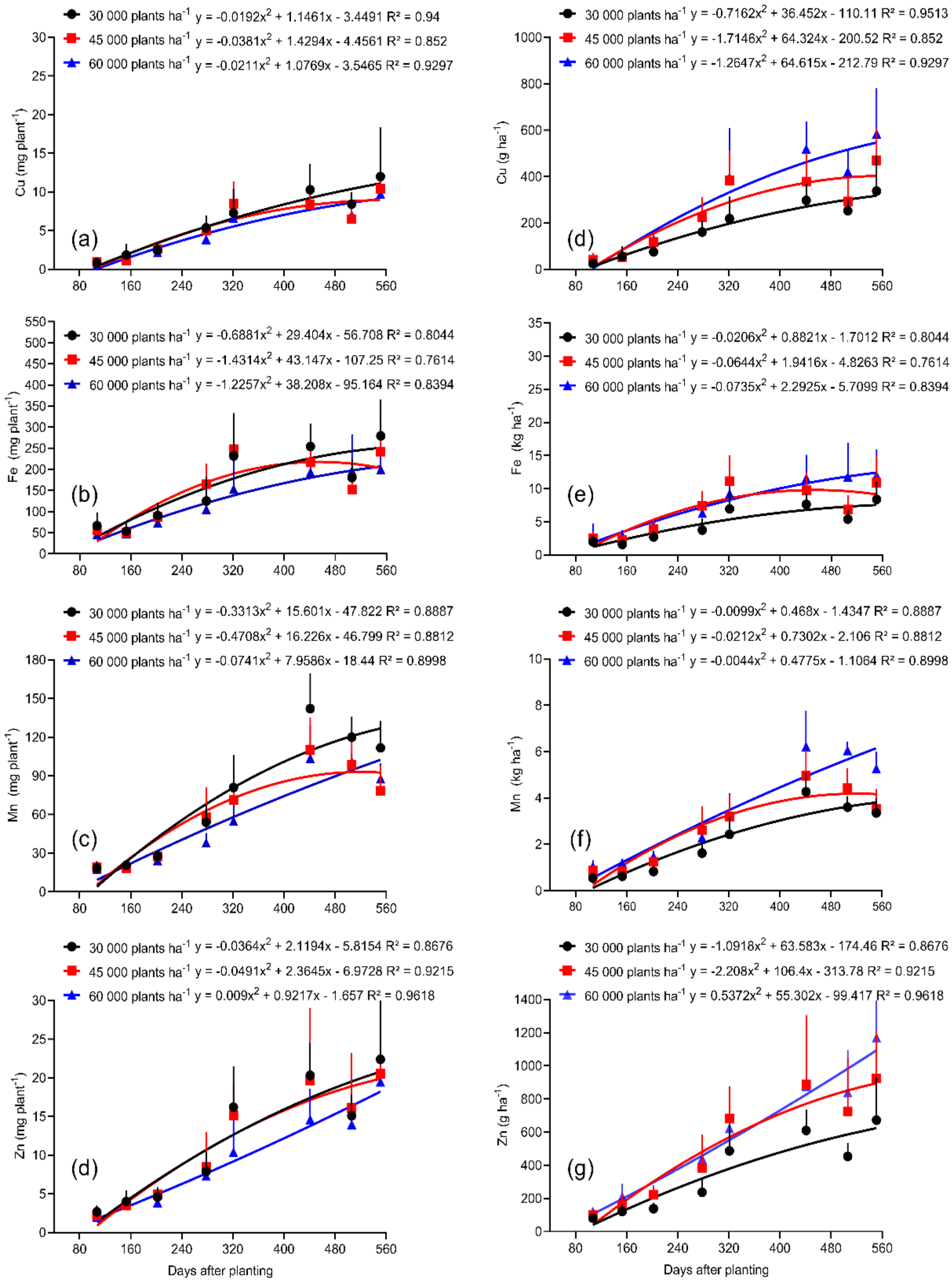

Figure 2. Copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) content in pineapple plants over time, as a function of three planting densities, in Isla, Veracruz, Mexico. The lines in the symbols correspond to the standard error.

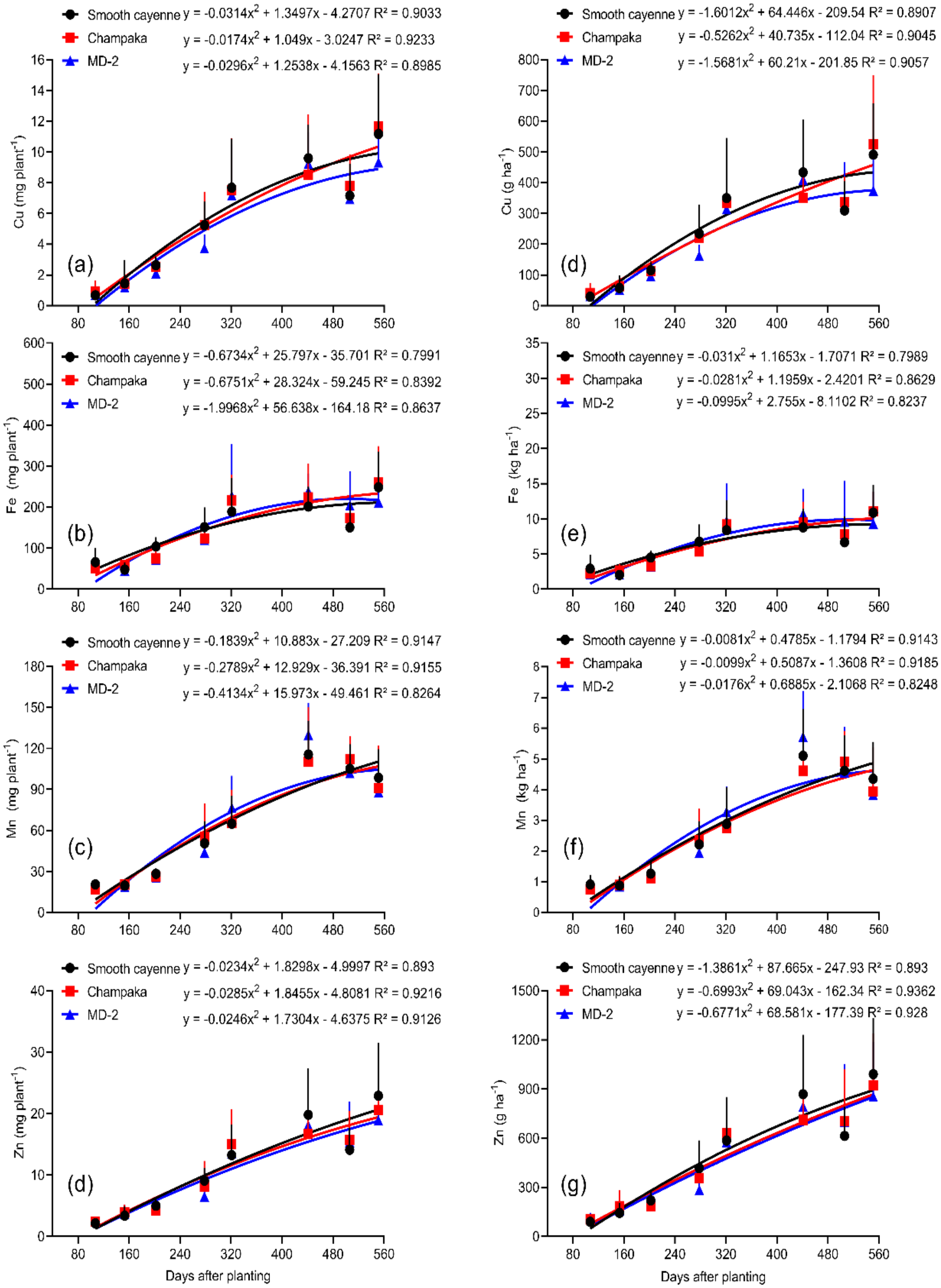

Figure 3. Copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) content in 'Smooth cayenne', 'Champaka', and 'MD-2' pineapple plants over time in Isla, Veracruz, Mexico. The lines in the symbols correspond to the standard error.

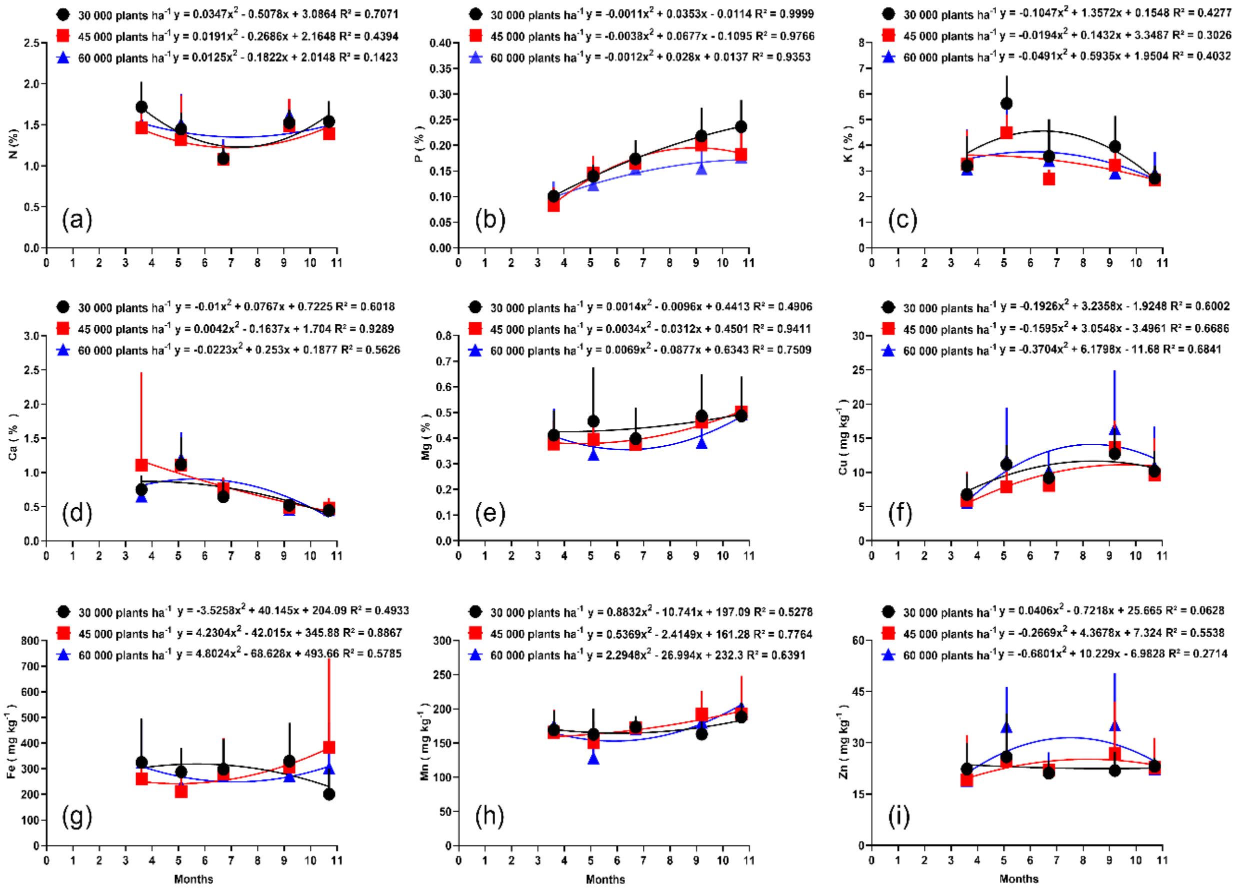

Figure 4. Concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in leaf D of pineapple plants over time, as a function of three planting densities, in Isla, Veracruz, Mexico. The lines in the symbols correspond to the standard error.

correlated with Cu and Mn content in two samples (Table 1). Leaf Fe concentration was correlated with Mn content in three samplings (one positive correlation and two negative correlations), two positive correlations with Cu content, three correlations with Mn content (two positive correlations and one negative correlation) and two negative correlations with Zn content (Table 1). Leaf Mn concentration was negatively correlated with Cu content in two samplings, and there were two correlations with Zn content (one positive and one negative), as well as one positive correlation with Fe content (Table 1). As for leaf Zn concentration, three correlations were detected with Fe content (two negative correlations and one positive correlation), two negative correlations with Zn contents and one positive correlation with Cu and Mn contents (Table 1). Total Cu and Fe content correlated positively with Mn content (Table 1). Of the 180 possible combinations (generated from the nine leaf concentrations, four nutrient contents and five samplings performed), only in 10 combinations did the correlation occur at the time of flower induction (at 310 DAP); the other correlations were detected prior to the flower induction treatment.

For 'Champaka' pineapple, correlations with a "r" greater than 0.5 were not detected between leaf N concentration and Cu, Fe, Mn, and Zn content (Table 2). Leaf P concentration was positively correlated with Fe content in four samples, while a positive correlation with Mn and Zn content was only detected in one sample (Table 2). Leaf K concentration was positively correlated with Fe content in two samples and positively correlated with Mn content in one sample (Table 2). On the other hand, the leaf Ca concentration was negatively correlated with Fe concentration in three samples and negatively correlated with Zn content in one sample (Table 1). Leaf Mg concentration was correlated with Fe content in two samplings (one positive correlation and one negative correlation) and it was negatively correlated with Cu content in one sampling (Table 2).

Table 1. Pearson correlation coefficient between total copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) contents in 'Smooth cayenne' pineapple plant with concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Cu, Fe, Mn, and Zn of leaf D, at 107, 153, 202, 278, and 321 days after planting (DAP).

	N					P				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	0.30	0.33	0.09	-0.32	-0.64	-0.10	-0.37	-0.32	-0.90	0.31
Fe total	-0.72	0.22	-0.77	-0.70	-0.43	-0.76	0.79	0.67	-0.32	-0.56
Mn total	0.01	0.82	0.00	-0.12	-0.20	-0.72	0.23	-0.17	-0.44	-0.13
Zn total	-0.14	-0.67	-0.44	-0.77	-0.86	0.40	-0.28	0.33	-0.76	0.24
	K					Ca				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	-0.44	0.69	0.46	0.44	-0.14	-0.14	-0.37	0.26	-0.49	-0.20
Fe total	0.56	-0.35	0.50	-0.16	-0.71	0.56	-0.65	-0.74	-0.19	-0.03
Mn total	0.33	0.00	0.18	0.16	-0.11	0.16	-0.86	-0.04	-0.28	-0.63
Zn total	-0.70	0.68	0.69	0.27	-0.51	-0.06	0.39	-0.15	-0.32	0.72
	Mg					Cu				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	-0.01	-0.68	0.80	-0.80	0.44	0.21	0.88	0.63	-0.33	0.07
Fe total	0.89	0.15	-0.23	0.09	-0.35	0.83	0.15	0.77	-0.68	-0.69
Mn total	0.60	0.04	0.14	-0.63	-0.33	0.60	0.35	0.55	-0.11	0.09
Zn total	-0.31	-0.87	0.70	-0.01	0.86	-0.01	0.74	0.58	-0.79	-0.57
	Fe					Mn				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	-0.45	0.77	-0.48	0.54	-0.29	-0.10	-0.87	0.13	-0.52	0.09
Fe total	-0.69	-0.24	-0.30	0.80	-0.43	0.88	-0.13	0.47	0.28	0.07
Mn total	-0.21	0.57	-0.85	0.95	0.02	0.18	-0.30	-0.20	-0.25	0.21
Zn total	-0.80	-0.02	0.49	-0.17	-0.77	0.24	-0.79	0.84	-0.15	-0.21
	Zn									
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	Cu total	Fe total	Mn total	Zn total	
Cu total	0.06	0.69	-0.12	-0.04	-0.04	1.00				
Fe total	0.83	-0.49	-0.70	-0.04	-0.79	0.26	1.00			
Mn total	0.61	0.06	-0.29	0.41	-0.07	0.69	0.62	1.00		
Zn total	-0.24	0.48	-0.20	-0.76	-0.54	0.47	0.13	-0.17	1.00	

Leaf Cu concentration was only positively correlated with Fe and Zn content in the first sampling (Table 2). Leaf Fe concentration was negatively correlated with Cu, Fe, and Zn content in one sampling (Table 2). Regarding the leaf Mn concentration, was correlated with Fe content in two samplings (one positive correlation and one negative correlation) and was positively correlated with Mn content in only one sampling (Table 2). Leaf Zn concentration was positively correlated with Mn and Zn content in one of the five samples taken (Table 2). Only the total Fe content was positively correlated with the total Zn content (Table 2). Of the 180 possible combinations (generated from the nine leaf concentrations, four nutrient contents and five samplings carried out), correlation occurred only in two combinations at the time of flower induction; the other correlations were detected prior to the flower induction treatment.

For pineapple 'MD-2', correlations with a "r" greater than 0.5 were detected between leaf N concentration with Cu, Mn, and Zn content in one sampling and with Fe content in two samplings (Table 3).

Table 2. Pearson correlation coefficient between total copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) contents in 'Champaka' pineapple plant with concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Cu, Fe, Mn, and Zn of leaf D, at 107, 153, 202, 278, and 321 days after planting (DAP).

	---------------------- N ----------------------					------------------------ P -----------------------				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	-0.08	-0.14	-0.42	0.46	0.23	0.23	0.35	0.29	-0.08	0.12
Fe total	-0.23	0.19	0.49	-0.32	-0.15	0.64	0.56	0.82	0.51	-0.10
Mn total	0.35	0.47	-0.34	0.00	-0.06	0.66	-0.28	0.29	0.45	0.22
Zn total	-0.34	0.14	-0.08	0.18	0.33	0.44	0.15	0.67	0.25	0.01
	---------------------- K ----------------------					---------------------- Ca ----------------------				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	-0.47	-0.44	-0.36	-0.08	-0.43	0.09	0.31	-0.32	-0.37	0.01
Fe total	0.28	0.55	0.01	0.64	0.01	0.19	-0.50	-0.80	-0.08	-0.50
Mn total	-0.02	0.43	0.52	0.45	0.39	-0.33	0.22	-0.44	0.24	-0.37
Zn total	-0.04	-0.19	-0.22	0.06	-0.45	-0.08	0.11	-0.73	-0.41	-0.30
	---------------------- Mg --------------------					--------------------- Cu ----------------------				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	-0.53	-0.28	0.18	0.19	0.40	-0.28	0.49	-0.39	-0.01	-0.31
Fe total	-0.04	0.10	-0.51	0.68	0.17	0.55	-0.19	-0.02	0.48	-0.20
Mn total	0.06	0.01	0.05	-0.15	0.02	0.63	0.48	0.10	-0.39	-0.24
Zn total	-0.06	-0.34	0.20	0.31	0.32	-0.10	0.35	-0.18	0.05	-0.37
	---------------------- Fe ---------------------					--------------------- Mn ---------------------				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	-0.47	-0.58	0.23	-0.41	0.09	0.19	-0.14	0.05	0.35	-0.04
Fe total	-0.53	-0.23	-0.05	0.29	0.06	-0.47	0.82	0.01	0.32	-0.59
Mn total	0.04	0.37	0.47	-0.07	-0.37	-0.44	0.20	0.55	-0.23	-0.20
Zn total	-0.35	-0.54	0.17	-0.32	-0.25	0.12	0.15	-0.13	0.37	-0.42
	-------------------- Zn ----------------------					---				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	Cu total	Fe total	Mn total	Zn total	
Cu total	-0.47	0.40	-0.08	-0.45	-0.21	1.00				
Fe total	0.45	-0.04	-0.40	-0.43	-0.30	0.14	1.00			
Mn total	0.60	0.29	-0.41	-0.42	-0.37	-0.06	0.31	1.00		
Zn total	-0.20	0.56	-0.26	-0.46	-0.39	0.80	0.47	0.21	1.00	

Leaf K concentration only promoted positive correlations with Cu content in one sampling and with Mn and Zn content in two samplings (Table 3). Regarding the leaf Ca concentration, it was correlated with Fe content in three samples (two positive correlations and one negative correlation), with Cu and Zn content in two samples (one positive correlation and one negative correlation) and with Mn content (Table 3). Leaf Mg concentration was correlated with Cu content in two samples (one positive correlation and one negative correlation) and positively correlated with Fe and Zn content in one sample (Table 3). Leaf Mn concentration was negatively correlated with Cu, Mn, and Zn content in one sampling, while a positive correlation with Fe content was detected in two samplings (Table 3). On the other hand, leaf Zn concentration was positively correlated with Cu content in one sampling, negatively correlated with Mn content in one sampling and correlated in two samplings with Fe content (one positive correlation and one negative correlation) (Table 3). No correlation was detected between leaf Fe concentration and Cu, Fe, Mn, and Zn content (Table 3). A positive correlation was detected between total Cu content and total Fe, Mn, and Zn content, and a positive correlation was detected between total Fe content and total Mn and Zn content (Table 3). Of the 180 possible combinations (generated from the nine leaf concentrations, four nutrient contents and five samplings carried out), correlation occurred only in two combinations at the time of flower induction; the other correlations were detected prior to the flower induction treatment (Table 3).

DISCUSSION

Independent of planting density and variety, the plant micronutrient content presented maximum uptake in the following order: Fe > Mn > Zn > Cu, which agrees with the compilations reported by Vázquez-Jiménez and Bartholomew (2018) and Maia *et al.* (2020). The maximum Fe, Mn, Zn and Cu uptake per hectare detected with the density of 30,000 plants ha-1 corresponded to 8.4, 4.3, 0.7 and 0.3 kg ha-1, whereas, with 60,000 plants ha-1, they corresponded to 12.7, 6.2, 1.2 and 0.6 kg ha^{-1}. These values differ with the reports of Hanafi *et al.* (2009), with a density of 62 000 plants ha^{-1} they found that the varieties 'Gandul', 'Moris', 'N-36', and 'Josapine' can absorb a higher amount of Fe (3.00 - 4.33 g $plant^{-1}$ and 186 - 268 kg ha^{-1}) and Cu (0.15 - 12.17 g $plant^{-1}$ and 9.3 - 138 kg ha^{-1}). It is important to note that more than 50% of Cu, Fe, Mn, and Zn have already been absorbed by the pineapple plant at the time of flower induction and this proportion increases between 81 and 95% at the

Table 3. Pearson correlation coefficient between total copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) contents in 'MD-2' pineapple plant with concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Cu, Fe, Mn, and Zn of leaf D, at 107, 153, 202, 278, and 321 days after planting (DAP).

	N					P				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	0.14	-0.71	-0.22	-0.27	0.21	0.03	0.03	0.38	-0.47	-0.09
Fe total	-0.64	-0.89	0.32	0.00	0.16	-0.56	0.38	0.63	-0.25	-0.56
Mn total	0.12	-0.68	-0.22	-0.26	0.32	-0.24	-0.06	0.23	-0.16	0.04
Zn total	0.02	-0.70	0.06	-0.23	0.16	-0.01	0.08	0.37	-0.45	-0.20
	K					Ca				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	0.35	0.06	0.45	0.81	0.37	-0.23	-0.59	-0.16	0.43	0.61
Fe total	0.03	-0.42	0.18	0.35	-0.32	0.08	-0.68	-0.56	0.58	0.45
Mn total	0.72	-0.01	0.06	0.71	-0.11	-0.18	-0.52	-0.44	0.33	0.16
Zn total	0.07	-0.19	0.63	0.65	0.40	-0.18	-0.52	-0.05	0.49	0.81
	Mg					Cu				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	0.17	-0.51	-0.13	0.27	0.57	-0.24	-0.06	0.55	0.08	0.18
Fe total	0.43	-0.28	0.23	0.70	0.07	0.36	0.27	0.14	0.62	0.01
Mn total	0.15	-0.21	0.33	0.40	0.03	0.00	0.45	0.29	-0.09	0.44
Zn total	0.23	-0.41	-0.37	0.16	0.69	0.04	-0.09	0.54	0.43	0.11
	Fe					Mn				
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP
Cu total	-0.09	0.35	0.31	-0.23	-0.17	0.13	-0.76	-0.01	0.19	0.24
Fe total	-0.21	-0.44	-0.11	0.22	0.49	0.62	-0.49	0.10	0.56	0.21
Mn total	0.19	0.16	0.27	-0.04	-0.27	0.16	-0.56	-0.10	-0.06	0.21
Zn total	-0.12	0.37	0.24	-0.37	0.08	0.26	-0.88	0.18	0.46	0.02
	Zn									
	107 DAP	153 DAP	202 DAP	278 DAP	321 DAP	Cu total	Fe total	Mn total	Zn total	
Cu total	-0.27	-0.44	0.07	0.04	0.51	1.00				
Fe total	0.32	-0.63	-0.46	0.55	-0.25	0.52	1.00			
Mn total	-0.04	-0.65	0.23	-0.11	0.47	0.79	0.56	1.00		
Zn total	0.04	-0.36	-0.05	0.40	0.32	0.85	0.54	0.48	1.00	

end of flowering. This behavior detected in the micronutrients was also detected in the absorption of N, P, and K (Rebolledo-Martínez *et al.*, 2023) and corroborates the recommendation of Rebolledo Martínez *et al.* (2016), who indicate that, 100% of fertilization should be concluded at the maximum at the anthesis stage, so that the nutrients can be absorbed by the pineapple plants at the time they require it.

Soil is a source of nutrients for plants; nutrients are absorbed mainly through the roots; in the case of pineapple, most of its roots are in the first 20 cm of depth (Inforzato *et al.*, 1968; Chopart *et al.*, 2015). At a depth of 20 cm and a bulk density of 1 g cm^{-3}, in one hectare of land, there are 2000 t of soil. Considering that the soil used in this research presented an exchangeable Fe, Mn, Zn, and Cu concentration of 50.00, 16.00, 0.90, and 1.23 mg kg^{-1}, at a depth of 20 cm, the amount available for plants was at least 100, 32, 1.8, and 2.6 kg ha^{-1}, respectively. According to the above, it is speculated that the micronutrients present in the soil were sufficient to meet 100% of the requirements for Fe, Mn, Zn, and Cu in the three pineapple varieties.

In Mexico, 73% of the soils cultivated with pineapple are in the Papaloapan Basin, in the state of Veracruz (SIAP, 2023), which are characterized by a pH of 4.5 to 5.2 (Zetina *et al.*, 2005). In this study, the soil pH was 4.8, with this pH, of the four micronutrients studied, Fe is the most available (Thapa *et al.*, 2021), which could explain its higher content in pineapple plants. Considering that the highest availability of Cu, Fe, Mn, and Zn occurs at a pH of 4.8 to 5.5 (Thapa *et al.*, 2021), it is expected that in soils with this characteristic and with an adequate distribution of precipitation, deficiencies of these micronutrients are not detected in plants. On the other hand, in soils with a pH below 4.8, liming is recommended to decrease acidity and achieve a pH of approximately 5.0, a value within the recommended range for pineapple (Huerta Uscanga *et al.*, 2019; Maia *et al.*, 2020).

If the availability of nutrients in the soil is unknown, foliar applications of Fe, Mn, Zn and Cu should be made to restore the edaphic nutrients extracted by the plants. In Mexico, for these nutrients, two foliar fertilizations are recommended at three and five months after planting. For each 100 L of water, it is recommended to apply 100 g of ferrous sulfate ($FeSO_4.7H_2O$, 21% Fe), 100 g of manganese sulfate ($MnSO_4.7H_2O$, 32% Mn), 50 g of zinc sulfate ($ZnSO_4$. $7H_2O$, 35% Zn) and 50 g of copper sulfate ($CuSO_4.7H_2O$, 13% Cu), together with 40 g of the chelating agent citric acid (Rebolledo Martínez *et al.*, 2016; Uriza-Ávila *et al.*, 2018). Considering that, for each foliar fertilization it is recommended to apply 50 mL of the solution per plant (Rebolledo Martínez *et al.*, 2016), with a density of 30 000 plants ha^{-1} a total of 0.63, 0.96, 0.53 and 0.20 kg ha^{-1} of Fe, Mn, Zn, and Cu are supplied. With these amounts, 10, 31, 88 and 65% of the total absorbed by the plant is supplied to the soil. When 60 000 plants ha^{-1} are used, with the two foliar fertilization a total of 1.26, 1.92, 1.05 and 0.39 kg ha^{-1} of Fe, Mn, Zn and Cu are added to the soil, with these amounts 10, 31, 88 and 65% of the total absorbed by the plant is returned to the soil. In case the producer wishes to use ferrous sulfate, manganese sulfate, zinc sulfate and copper sulfate, according to the results of this study, to restore 100% of Fe, Mn, Zn, and Cu, for a density of 30,000 plants ha^{-1} a total of 40.0, 13.4, 2.0 and 2.3 kg ha^{-1} should be applied. For a density of 60,000 plants ha^{-1}, this total increases to 60.5, 19.4, 3.4 and 4.6 kg ha^{-1}, respectively. These total amounts can be divided according to the producer's needs, considering volumes of 1500 and 3000 L of water for densities of 30,000 and 60,000 plants ha-1, respectively. For pineapple 'MD-2', the total Cu can be reduced by 30% because the maximum amount absorbed was lower compared to 'Smooth cayenne' and 'Champaka'.

In the case of leaf nutrient concentration, it was not possible to compare our results with similar studies conducted in Mexico; therefore, these values will serve as a point of comparison for future research. Comparing our results with reports from other producing regions and other pineapple varieties, N, P, Cu and Zn were classified closer to a deficiency condition. According to the compilation of Uriza-Ávila *et al.*, (2018), the optimum concentrations of N, P, Cu and Zn are 1.4 - 2.5%, 0.1 - 0.34%, 10 - 50 mg kg^{-1} and 20 - 70 mg kg^{-1} and, in this study those values were 1.1 - 1.7%, 0.08 - 0.23%, 6 - 16 mg kg^{-1} and 19 - 35 mg kg^{-1}, respectively. According to the same authors, the concentrations of Mg (0.33 - 0.49%) and Mn (128 - 202 mg kg^{-1}) were classified as adequate. The maximum value of K, Ca and Fe considered adequate are 4.5%, 0.8% and 200 mg kg^{-1} (Uriza-Ávila *et al.*, 2018). In this study, the concentrations of K, Ca and Fe were 2.64 - 5.62%, 0.45 - 1.20% and 200 - 383 mg kg^{-1}. Therefore, they are classified between optimal and close to a toxic condition. This applies mainly to Ca and Fe, since they exceeded the optimum range at all three planting densities. At the same time, K was only detected at a density of 30,000 plants ha^{-1}, precisely five months after planting.

To assess the nutritional status of the pineapple plant during its growth, the primary tool used is foliar analysis on leaf D, as it is the youngest of the adult leaves and physiologically the most active (Queiroga *et al.*, 2023). The foliar analysis at the time of the floral induction treatment (±15 days before or after) has been used as a reference (Souza, 2000), although it has also been recommended to conduct more than one analysis to make pertinent adjustments to the fertilisation program (Uriza-ávila *et al*., 2018). The results of the study indicate that performing multiple leaf analyses is a prudent approach. In the three pineapple cultivars, a high Pearson correlation (≥ 0.5) between leaf nutrient concentration and total Fe, Mn, Zn and Cu content at harvest was only detected between 2 and 10 occasions, out of the 180 possible combinations generated from sampling dates, leaf nutrient concentrations, and nutrient contents. Considering the three pineapple varieties and the five sampling dates, a high correlation was detected between concentrations and micronutrient content on 72 occasions. Of this total, 21, 22, 19, 19, and 18% corresponded to sampling carried out at 107, 153, 202, 278, and 321 days after planting. Based on the above, it can be inferred that the probability of achieving adequate nutrition increases when more than one leaf analysis is performed during the plant's vegetative development.

The correlation analysis also indicated that the effect of leaf nutrient concentration on total content changes depending on the type of micronutrient. When considering the three pineapple varieties, a high correlation was found 135 times, with correlations of 40%, 25%, 17%, and 18% with the total content of Fe, Zn, Mn, and Cu, respectively. Based on the above, it can be deduced that, as a diagnostic tool for detecting and correcting nutrient deficiencies, leaf analysis is more effective in predicting the behaviour of Fe and Zn, while it is less effective in predicting the behaviour of Mn and Cu. This indicates that, for Mn and Cu, in addition to their concentration in leaf D, the total content depends on other factors not considered in this analysis. On the other hand, the results indicate that the interaction of nutrients within the plant, in some cases, promoted an antagonistic effect and in other instances a synergistic effect. For example, of the total correlations classified with high correlation, considering the three pineapple varieties, the leaf concentration of N was the one that showed an antagonistic effect, since it was negatively correlated with the total content of Cu, Fe, and Zn. A completely synergistic effect occurred with the leaf concentration of K, Mg, and Zn, since, as the leaf concentration of these nutrients increased, the total content of Cu, Fe, Mn, and Zn also increased. As for the leaf concentration of P, Ca, Fe, and Mn, the tendency was more towards a synergistic effect, since they were positively correlated with the total content of three of the four micronutrients evaluated. In pineapple 'MD-2', Valleser (2019) found that, with a P fertilization rate above 169 kg ha^{-1}, P presents an antagonistic effect with Zn. For this study, at the three planting densities, the P applied was more than 200 kg ha-1; however, a completely antagonistic effect was only detected with Cu, and a moderately antagonistic effect was observed with Mn and Zn (in 50% of the correlations). On the other hand, Vásquez Jiménez (2010) in pineapple 'MD-2' indicates an antagonistic effect between Fe and Mn, which partially coincides with what was found in this study, as a high negative correlation between these micronutrients was only detected on 40% of the occasions. No other similar study was found to compare the results; therefore, these findings will serve as a reference point for future research in Mexico.

CONCLUSIONS

It is concluded that the amount of Fe, Mn, Cu, and Zn extracted increases as planting density increases, reaching maximum values of 12.7, 6.2, 1.2, and 0.6 kg ha^{-1}. The Fe extraction of the ‘MD-2’ variety is 30% lower than that of ‘Smooth cayenne’ and ‘Champaka’, therefore, the first hypothesis is partially accepted. A high correlation between leaf nutrient concentration and total Fe, Mn, Cu, and Zn content was only detected in 18% of the occasions during the flower induction stage (at 10.1 months after planting). A high correlation was detected 82% of the time in samples taken between 153 and 321 days after planting, therefore, the second hypothesis of the study is partially accepted. Additionally, it was found that leaf analysis in D leaf as an optimal diagnostic tool for nutrition, it is effective for predicting the behavior of Fe (217 a 340 mg kg^{-1}), Zn (19 a 34 mg kg^{-1}), Mn (146 a 192 mg kg^{-1}) and Cu (4.6 a 13 mg kg^{-1}) from the fourth month after planting until flowering induction.

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