Balanced Mineral Nutrition Decreases Greasy Spot Incidence in Citrus

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Diseases are a serious threat to profitable yields and quality of citrus. Disease control solely by continuous use of agrochemicals is not an acceptable, sustainable option due to environmental contamination, residues in the food chain, and potential development of pathogen resistance. Therefore, alternative integrated pest management (IPM) techniques need to be developed and evaluated. Mineral nutrition could increase or decrease disease resistance by affecting both plant and pathogen growth. To evaluate the effect of mineral nutrition on greasy spot disease incidence in citrus, a greenhouse hydroponics experiment was established for precise control of nutrient elements. 'Valencia' orange plants [*Citrus sinensis* (L.) Osb.] were grown in plastic pots filled with graded quartz sand automatically drip-irrigated with deionized water. The experiment was conducted in a completely randomized design (CRD) with seven nutritional treatments and 10 replications. The treatments included full-strength Hoagland's nutrient solution (T1), one-tenth concentration Hoagland's nutrient solution (T2), full-strength Hoagland's solution minus Mg (T3), minus Ca (T4), minus B (T5), minus Mn (T6), or minus Zn, Cu, Mo, and Fe (T7). The nutrient solution specific to each treatment was applied weekly. A high humidity was maintained in the greenhouse to encourage greasy spot spore germination and natural leaf infection. Greasy spot incidence was up to 90% lower in the full-strength balanced nutrient treatment (T1) than in the nutrient-deficient treatments. Electrolyte leakage, which is an indicator of compromised cell membrane integrity, was higher in T2 and T4 than in the full-strength nutrient treatment. Leaf sap pH was lower in different nutrient-deficient treatments (pH 6.0–6.2) than in the balanced nutrition treatment (pH 6.3). Leaf nutrient concentrations, principal component analysis (PCA), and canonical discriminant function analysis (DFA) revealed that omitting any one of the nutrients can increase susceptibility to greasy spot. The results confirm Liebig's law of minimum, and suggest that a complete balanced nutrition supply for citrus may significantly reduce the occurrence of greasy spot, reduce pesticide spray requirements, promote overall tree health, and enhance production efficiency.

Plant diseases are the primary limitation to crop production and continue to be a major concern due to the large economic losses. Citrus is frequently subjected to a number of diseases and pests, which reduce yield, fruit quality, and profitability. Greasy spot caused by *Mycosphaerella citri* Whiteside (Whiteside, 1972) is a serious disease of citrus. *M. citri* produces yellow to dark brown lesions on leaves, causes yellowing and premature defoliation of leaves and a rind blemish on fruit, reducing the exterior quality, yield, and marketability (Burnett and Whiteside, 1983; Whiteside, 1972).

Greasy spot in citrus is primarily controlled by copper (Cu), petroleum oils, benomyl, fenbuconazole, or strobilurin-containing fungicides (Dewdney and Timmer, 2010; Timmer and Zitko, 1995; Whiteside, 1983); however, these chemicals can be harmful to the environment. For example, Cu can accumulate to toxic levels in soil if the pH is below 6.0–6.5 (Reuther and Smith, 1954b). Benomyl was found to be very effective at suppressing greasy spot symptoms, but resistance development was problematic (Whiteside, 1983). The major challenges of using agrochemicals are the risk of environmental contamination, chemical residues in the food chain, and resistance development (Yadav, 2010). Thus, continuous use of agrochemicals is not a sustainable option. Alternatively, integrated pest management (IPM) techniques like biological control, host plant resistance, cultural control and balanced mineral nutrition need to be developed and evaluated.

Pathogens can alter the plant's physiology, and reduce nutrient uptake, distribution, or utilization by the plant (Huber, 1980). They can cause malfunctioning of the vascular system and change the permeability of cell membranes (Cantu et al., 2008). Increased permeability can result in the loss of nutrients through root or leaf exudation, attract pathogens, or enhance infection (Huber and Haneklaus, 2007). Mineral nutrition can increase plant resistance and reduce disease incidence either by their direct toxic effect on the pathogen and by reducing susceptibility of the host, or by enhancing the population of antagonistic members of the soil microflora (Huber and Watson, 1974). Nutrients can support increased cell wall thickness as a mechanical barrier or synthesis of natural defense compounds as a protection against pathogens (Rice, 2007). The interaction of mineral nutrition with plant diseases is dynamic and all essential nutrients usually influence the disease incidence or severity in one or another.

Calcium (Ca), zinc (Zn), and boron (B) have significant roles in maintaining structural integrity and controlling permeability of cell membranes. In addition, nutrients are responsible for production of antimicrobial compounds like phytoalexins, phenols,

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flavonoids, and other defense compounds (Huber, 1980; Maathuis, 2009). The role of mineral nutrition in disease control has long been recognized in a variety of crops, including wheat, cabbage, tomato, cucumber, maize, rose, grape, apple, and mango (Huber and Haneklaus, 2007; Reuveni and Reuveni, 1998; Walker, et al., 1954). Foliar applications of various mineral nutrient products containing N, Zn, Mn, and Fe are reported to be very effective against greasy spot symptoms in citrus (Timmer and Zitko, 1995). Despite the known beneficial effects of mineral nutrition in preventing disease occurrence, it is often ignored as a component of IPM programs for sustainable crop production.

Although specific nutrients have been associated with the control of diseases, a balance between all of the essential nutrients is also important. For example, the effect of K in reducing disease susceptibility and development is related to its interaction with Ca and Mg (Huber, 1980). Also, K decreases take-all disease of wheat if N and P are sufficient, but increases this disease if they are deficient (Huber and Haneklaus, 2007). An imbalance in the nutrient management and ignorance of the law of minimum creates a more favorable environment for pest attack and disease incidence (Liew et al., 2010). Therefore, balanced mineral nutrition is the key for maintaining a high level of disease resistance and producing healthy sustainable crops. However, interactions between plants, nutrients, and pathogens are very specific, complex and not completely understood. Moreover, plant nutrient deficiency due to variable soil properties can also affect disease occurrence, and plants suffering a nutrient stress due to variable soil properties are less vigorous and more susceptible to diseases (Broders et al., 2009).

The basis for nutrient applications to crops as a protection from pathogens is to avoid plant stress so that crops can better withstand pathogen attack, and to manipulate nutrients for the advantage of the plant and disadvantage of the pathogen. Therefore, a thorough understanding of disease interaction with each specific nutrient can help modify the plant's environment to improve disease control. Hence, the objectives of our study were to 1) determine the role of balanced mineral nutrition in controlling greasy spot disease and 2) evaluate the relationships between specific mineral nutrients and greasy spot incidence in citrus.

Materials and Methods

Plant material and experiment treatments

Seventy 'Valencia' sweet orange trees [*Citrus sinensis* (L.) Osb.] on Carrizo citrange rootstock [*C. sinensis* L. Osb. × *Poncirus trifoliata* (L.) Raf.] were purchased from a commercial citrus nursery. The trees were approximately 15 months old when purchased and had been grown in 2.65-L citra-pots (model CPOT-5H, Stuewe and Sons, Inc., Tangent, OR) using a standard peat, pine bark, and perlite potting media. The trees were removed from their containers, the roots washed free of potting media, and replanted into 12.04-L plastic pots (model TP915R, Stuewe and Sons, Inc.) with a graded quartz sand. The trees were moved to a greenhouse with daily mean temperature from 18 to 24 °C, relative humidity from 74% to 87%, and natural photoperiod. The trees were automatically drip-irrigated using deionized water.

The experiment was conducted in a completely randomized design (CRD) with seven nutritional treatments and 10 replications. The treatments included full-strength Hoagland's nutrient solution (T1) used as a standard control, one-tenth concentration Hoagland's nutrient solution (T2), full-strength Hoagland's minus magnesium (Mg) (T3), minus calcium (Ca) (T4), minus boron (B) (T5), minus manganese (Mn) (T6), or minus zinc (Zn), copper (Cu), molybdenum (Mo), and iron (Fe) combined (T7). The Hoagland's nutrient solution consisted of 210 $mg \cdot L^{-1}$ nitrogen (N), 31 $mg \cdot L^{-1}$ phosphorus (P), 235 $mg \cdot L^{-1}$ potassium (K), 200 $mg \cdot L^{-1}$ Ca, 48 $mg \cdot L^{-1}$ Mg, 119 $mg \cdot L^{-1}$ sulfur (S), 0.5 $mg \cdot L^{-1}$ B, 0.05 $mg \cdot L^{-1}$ Zn, 0.5 $mg \cdot L^{-1}$ Mn, 3 $mg \cdot L^{-1}$ Fe, 0.02 $mg \cdot L^{-1}$ Cu, and 0.01 $mg \cdot L^{-1}$ Mo. The nutrient solutions were derived from $(NH_4)_2SO_4$, NH_4NO_3, KH_2PO_4, K_2SO_4, KCl, $MgSO_4.7H_2O$, $Ca(NO_3)_2 \cdot 4H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, H_3BO_3, $CuSO_4 \cdot 5H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$+Na-EDTA. The nutrient solution specific to each treatment was applied to the sand surface weekly at 500 mL per tree.

The greenhouse temperature and humidity were optimum for maximum ascospore development of greasy spot fungus (Mondal and Timmer, 2002), which was allowed to naturally enter the greenhouse from the surrounding orchards through the ventilation system. The greasy spot symptoms appeared on the lower leaves; therefore, the lowest 20 leaves of each tree were used for assessing disease occurrence and other measurements.

Disease assessment

Disease symptoms were assessed biweekly from Jan. 2011 to Mar. 2011 after the onset of greasy spot in Dec. 2010. Disease assessment was recorded both as disease incidence and disease severity. Disease incidence was recorded as the number of infected leaves out of 20 leaves per tree and expressed as percent incidence. Greasy spot incidence was rated on a scale of 0 = none; 1 = 1% to 5%; 2 = 6% to 10%; 3 = 10% to 15%; 4 = 16% to 20%; and 5 = >20% of the leaf surface area affected by the disease (Timmer et al., 2000). Disease severity was rated on a scale of 1 = no infection; 2 = <25% infection; 3 = 25% to 50% infection; 4 = 50% to 75% infection; 5 = 75% to 100% infection; 6 = defoliated leaves.

Measurements

Chlorophyll index was measured using a SPAD-502 chlorophyll meter (Minolta Corp., Ramsey, NJ). The measurements were taken on five fully mature leaves out of the lower 20 leaves for which the disease was assessed. Stem diameter was measured 3 cm above the bud union with calipers. Ten fully expanded recently matured leaves were collected for nutrient analysis. Leaf samples were washed, dried at 70 °C for 48 h and ground to pass a 0.38-mm sieve. Leaf P, K, Ca, Mg, S, Mn, Fe, Cu, Zn, B, and Mo concentrations were determined by inductively coupled plasma atomic emission spectroscopy with a method described by Isaac and Johnson (1985).

To look at the effect of leaf position on electrolyte leakage, pH, and Brix contents, leaves were collected from different positions of the tree. Out of the lower 20 leaves from each plant, one additional leaf sample was taken from each of the top, middle, and lower positions. Detached leaves were washed with deionized water, dried with soft paper towel, and placed on moist filter paper in glass petri dishes. The leaves in petri dishes were exposed to 50 °C heat stress for 5 h then kept at room temperature for a 16-h recovery period. Electrolyte leakage, pH, and Brix were determined for all samples before and after heat stress treatment. Thus, total experimental units for laboratory measurements were 420 (seven nutritional treatments × 10 greenhouse replications × three locations × two types of stress conditions—heat and no heat).

Electrolyte leakage percentage was used to assess membrane permeability according to the method described by Sairam et al. (1997). Briefly, five, 10-mm leaf discs were cut from each leaf segment. Leaf discs were placed into glass vials containing 15 mL DI water, covered and placed in an oven at 40 °C for 30 min. After incubation, the samples were stirred, allowed to cool to room temperature and electrical conductivity (EC1) was measured. The vials were then placed in a water bath at 95 °C for 20 min, allowed to cool to room temperature, and electrical conductivity measured (EC2). Electrolyte leakage was calculated as the ratio of EC1 to EC2 and expressed as a percent. After measuring electrolyte leakage, Brix content of the solution was measured using a Digital Palette Portable Brix/Sucrose Refractometer (National Microscope Exchange, Carnation, WA) and the solution pH was measured using a Corning 450 (Corning, Inc., Corning, NY) pH meter.

Statistical analysis

Analysis of variance (ANOVA) was performed and means were separated using Fisher's protected least significant difference (LSD) at 5% confidence interval. For the laboratory measurements of electrolyte leakage, pH, and Brix, the interaction effect (treatment × leaf position) was nonsignificant; therefore only the main effects of treatment and leaf position are presented. All data were analyzed using SAS statistical software (SAS Institute, Inc., 2003). Correlation analysis was performed using PROC CORR. Multivariate principal component analysis (PCA) was performed using PROC PRINCOMP and canonical discriminant function analysis (DFA) was performed using PROC CANDISC. Wherever required the data were transformed to validate the ANOVA assumption of normality and then subjected to statistical analysis. Original data are presented in the tables and figures.

Results

Greasy spot incidence 2 weeks after the appearance of symptoms was up to 90% lower in the full-strength balanced nutrient treatment (T1) than in the nutrient-deficient treatments (Table 1). The disease severity was also significantly lower in T1 than in all other treatments except T5 (minus B). The treatment differences for disease incidence and severity at week 4 were similar to those at week 2; however, the treatment with omission of Mn (T6) also had highest severity at week 4.

All the treatments had greater (8.46–9.90 mm) stem diameter than the one-tenth concentration Hoagland's nutrient solution (T2), which averaged 6.94 mm (Table 2). Chlorophyll index ranged from 74 to 81 in all the treatments except T2 and T4 (full-strength Hoagland's minus Ca), which were 56 and 62, respectively.

Electrolyte leakage, pH, and Brix data before and after exposing the leaves to heat stress are presented separately because the interaction effect (treatment × heat stress) was significant (Table 3). Pre-heat stress electrolyte leakage did not show any statistical differences among treatments. The pH was higher in the full-strength nutrient treatment (6.3) than in the imbalanced nutrient treatments in which one or more nutrients were omitted. The lowest pH (6.0) was measured in the treatments supplied with one-tenth Hoagland's solution, minus Ca and minus B. Although the Brix content varied only between 0.0% and 2.4%, it differed significantly among treatments and the treatment with no Ca had the highest Brix content. Post-heat stress electrolyte leakage was significantly lower in the full-strength Hoagland's treatment compared with the one-tenth Hoagland's treatment.

We also looked at the effect of leaf position (lower, middle

Table 1. Greasy spot incidence and severity on 'Valencia' sweet orange trees grown under different nutritional treatments in a hydroponics experiment.

	Week after appearance of symptoms			
	2 Week		4 Week	
Treatment	Incidence[z] (%)	Severity[y] (1–6)	Incidence(%)	Severity (1–6)
Full-strength Hoagland's solution (T1)	17.5 ± 3.12 D[x]	1.6 ± 0.10 C	20.5 ± 3.27 D	3.1 ± 0.31 C
Full-strength/10 (T2)	89.5 ± 4.18 A	4.5 ± 0.32 A	95.0 ± 3.71 A	5.0 ± 0.21 A
Full-strength – Mg (T3)	53.0 ± 4.98 C	3.3 ± 0.26 AB	54.5 ± 6.61 C	4.8 ± 0.20 A
Full-strength – Ca (T4)	90.0 ± 6.79 A	3.8 ± 0.38 AB	94.0 ± 3.77 A	5.2 ± 0.25 A
Full-strength – B (T5)	73.5 ± 6.39 BC	1.7 ± 0.12 C	78.0 ± 21.6 B	3.5 ± 0.37 BC
Full-strength – Mn (T6)	78.5 ± 5.87 AB	3.2 ± 0.24 B	84.0 ± 5.11 AB	4.4 ± 0.37 A
Full-strength – Zn, Cu, Mo, Fe (T7)	79.0 ± 6.18 AB	3.3 ± 0.15 AB	87.0 ± 4.01 AB	4.2 ± 0.36 AB

[z]Disease incidence was based on the number of infected leaves.
[y]Disease severity on the lower 20 leaves were rated on the scale of 1 = no infection; 2 = 0% to 25% infection; 3 = 25% to 50% infection; 4 = 50% to 75% infection, 5 = 75% to 100% infection, 6 = leaves abscised.
[x]Means within columns followed by the same letter are not significantly different ($P > 0.05$) (n = 10). The ANOVA for disease incidence and severity was performed on the transformed data and original data are presented in the table.

Table 2. Stem diameter and chlorophyll index for 'Valencia' sweet orange trees grown under different nutritional treatments in a hydroponics experiment.

Treatment	Stem diam (mm)	Chlorophyll index
Full-strength Hoagland's solution (T1)	9.55 ± 0.32 AB[z]	80.97 ± 1.55 A
Full-strength/10 (T2)	6.94 ± 0.34 C	55.98 ± 2.92 C
Full-strength –Mg (T3)	9.45 ± 0.32 AB	74.06 ± 2.26 AB
Full-strength – Ca (T4)	9.47 ± 0.27 AB	62.17 ± 2.57 C
Full-strength – B (T5)	9.90 ± 0.32 A	74.59 ± 3.56 AB
Full-strength – Mn (T6)	9.46 ± 0.17 AB	74.71 ± 5.29 AB
Full-strength – Zn, Cu, Mo, Fe (T7)	8.46 ± 0.42 B	71.83 ± 3.17 B

[z]Means within columns followed by the same letter are not significantly different ($P > 0.05$) (n = 10).

and top) on electrolyte leakage, pH, and Brix content (Table 3). After the heat stress, there was more electrolyte leakage in leaves at the lower and top positions than the middle position. Opposite to this, leaf sap pH was lower in the leaves at lower position than the middle and top positions.

Plant nutrient concentrations also differed significantly between nutritional treatments (Table 4). The full-strength Hoagland's treatment had higher N content (2.2%) than the one tenth of full-strength treatment (1.8%). Leaves from the treatment in which Mg was omitted had lower P, Mg, Ca, Zn, Mn, Cu, and Mo than the full-strength treatment. The nutritional treatment without Ca had lower Mg, Ca, Mn, Fe, and Cu than the full-strength treatment T1. As expected, full-strength Hoagland's minus B (T5) had the lowest B; however, B was highest in the treatments in which micronutrients were omitted (T6 and T7). Full-strength Hoagland's minus Mn had the lowest Mn concentration.

The correlation analysis of greasy spot occurrence with select plant growth parameters showed that chlorophyll index, leaf sap pH, N, Fe, and Cu were negatively correlated to the disease occurrence and the relationships were positive with Mo (Table 5).

For the PCA, the first, second and third principal components (PC) explained 51%, 29%, and 13% of the variance between treatments, respectively (Table 6). In PCI, all nutrients had positive loading factors except N and Fe and there was a positive

Table 3. Electrolyte leakage, pH and Brix before and after the heat stress for leaves from 'Valencia' sweet orange trees grown under different nutritional treatments and for leaves at different positions of in the plant canopy in a hydroponics experiment.

	Before heat stress			After heat stress		
Treatment	Electrolyte leakage (%)	pH	°Brix	Electrolyte leakage (%)	pH	°Brix
Full-strength Hoagland's solution (T1)	8.86 ± 0.28 [NS]	6.30 ± 0.017 A[z]	0.16 ± 0.012 B	54.7 ± 4.19 BC	6.42 ± 0.02 A	0.14 ± 0.012 B
Full-strength /10 (T2)	9.13 ± 0.15	6.05 ± 0.016 D	0.10 ± 0.012 C	66.0 ± 3.82 A	6.16 ± 0.017 CD	0.02 ± 0.007 D
Full-strength – Mg (T3)	8.36 ± 0.47	6.11 ± 0.018 C	0.00 ± 0.00 D	51.2 ± 3.66 C	6.21 ± 0.018BC	0.00 ± 0.00 D
Full-strength – Ca (T4)	8.76 ± 0.16	6.02 ± 0.03 D	0.26 ± 0.01 A	61.3 ± 4.10 AB	6.13 ± 0.018 D	0.24 ± 0.015 A
Full-strength – B (T5)	8.81 ± 0.12	6.03 ± 0.014 D	0.00 ± 0.00 D	53.2 ± 4.01 BC	6.13 ± 0.016D	0.00 ± 0.00 D
Full-strength – Mn (T6)	7.39 ± 0.36	6.24 ± 0.014 B	0.17 ± 0.011 B	54.5 ± 4.16 BC	6.24 ± 0.02 B	0.11 ± 0.015 C
Full-strength – Zn, Cu, Mo, Fe (T7)	9.38 ± 0.63	6.19 ± 0.013 B	0.00 ± 0.00 D	56.2 ± 3.34 ABC	6.24 ± 0.014 B	0.00 ± 0.00 D
Position of the leaves						
Lower	9.07 ± 0.23 [NS]	6.09 ± 0.019 B	0.94 ± 0.012 [NS]	65.0 ± 3.49 A	6.16 ± 0.013 B	0.07 ± 0.01 [NS]
Middle	8.39 ± 0.34	6.16 ± 0.016 A	0.11 ± 0.011	45.2 ± 4.53 B	6.24 ± 0.02 A	0.08 ± 0.006
Top	8.54 ± 0.19	6.15 ± 0.013 A	0.94 ± 0.012	60.0 ± 5.19 A	6.25 ± 0.013 A	0.08 ± 0.05

[z]Means within columns followed by the same letter are not significantly different ($P > 0.05$) (n = 30 for the main effects of seven nutritional treatments and n = 70 for the main effects of three positions of the leaves).
[NS]Nonsignificant at $P > 0.05$.

Table 4. Plant nutrient concentrations for leaves of 'Valencia' sweet orange trees grown under different nutritional treatments in a hydroponics experiment.

	Plant nutrients (%)						
Treatment[z]	N	P	K	Mg	Ca	S	B
T1	2.20 ± 0.06 AB[y]	0.18 ± 0.02 BC	2.34 ± 0.12 BC	0.30 ± 0.01 B	2.93 ± 0.09 A	0.37 ± 0.05 [NS]	84.53 ± 5.00 B
T2	1.80 ± 0.12 C	0.29 ± 0.015 A	2.41 ± 0.07 BC	0.29 ± 0.01 B	3.18 ± 0.31 A	0.26 ± 0.02	84.44 ± 8.10 B
T3	2.18 ± 0.09 AB	0.13 ± 0.003 D	2.61 ± 0.11 AB	0.03 ± 0.002 D	2.30 ± 0.10 CD	0.27 ± 0.02	85.20 ± 2.45 B
T4	2.33 ± 0.09 A	0.17 ± 0.015 BCD	2.28 ± 0.11 C	0.25 ± 0.012 C	2.04 ± 0.07 D	0.35 ± 0.04	71.61 ± 3.43 B
T5	2.32 ± 0.04 A	0.16 ± 0.01 CD	2.33 ± 0.13 BC	0.28 ± 0.01 BC	2.50 ± 0.11 BC	0.31 ± 0.01	46.82 ± 1.92 C
T6	2.02 ± 0.08 BC	0.21 ± 0.02 B	2.62 ± 0.15 AB	0.30 ± 0.01 B	2.43 ± 0.09 BCD	0.41 ± 0.03	101.20 ± 7.27 A
T7	2.16 ± 0.05 AB	0.20 ± 0.014 BC	2.86 ± 0.07 A	0.35 ± 0.012 A	2.80 ± 0.04 AB	0.44 ± 0.04	112.26 ± 5.56 A

	Plant nutrients ($mg \cdot kg^{-1}$)				
Treatment	Zn	Mn	Fe	Cu	Mo
T1	17.74 ± 1.23 B	20.63 ± 3.00 AB	82.99 ± 3.58 A	4.13 ± 0.13 A	1.16 ± 0.58 BCD
T2	22.81 ± 2.32 A	16.76 ± 2.40 BC	65.64 ± 2.82 B	3.33 ± 0.21 B	3.00 ± 0.35 A
T3	11.01 ± 0.63 C	16.30 ± 1.47 BCD	80.41 ± 4.79 A	2.81 ± 0.12 C	0.41 ± 0.10 D
T4	16.85 ± 2.04 B	15.00 ± 1.26 CD	65.75 ± 4.88 B	2.66 ± 0.15 C	1.76 ± 0.25 B
T5	16.94 ± 1.89 B	18.08 ± 1.50 ABC	76.54 ± 4.18 AB	2.73 ± 0.12 C	0.59 ± 0.14 CD
T6	17.07 ± 1.09 B	11.38 ± 1.11 D	68.20 ± 3.07 B	2.96 ± 0.23 BC	1.30 ± 0.36 BCD
T7	19.81 ± 1.04 AB	21.91 ± 1.84 A	85.25 ± 4.00 A	4.280 ± 0.17 A	1.38 ± 0.24 BC

[z]The treatments included full-strength Hoagland's nutrient solution (T1), one-tenth concentration Hoagland's nutrient solution (T2), full-strength Hoagland's solution minus Mg (T3), minus Ca (T4), minus B (T5), minus Mn (T6), and minus Zn, Cu, Mo, Fe (T7).
[y]Means within columns followed by the same letter are not significantly different ($P > 0.05$) (n = 10).
[NS]Nonsignificant at $P > 0.05$.

Table 5. Correlation of greasy spot incidence and severity with selected plant growth parameters of 'Valencia' sweet orange trees grown under different nutrient treatments in a hydroponics experiment.

Disease occurrence	Chlorophyll index	Leaf sap pH	N	Fe	Cu	Mo
Disease incidence	–0.448***	–0.567***	–0.102NS	–0.276*	–0.284*	0.354**
Disease severity	–0.359**	–0.334**	–0.266*	–0.238*	–0.102NS	0.493***

NS, *, **, ***Nonsignificant at 0.05 probability level, and significant at $P < 0.05$, 0.01 and 0.001, respectively; n = 70.

Table 6. Principal component (PC) loadings of plant nutrients that significantly contributed to greasy spot incidence on 'Valencia' sweet orange trees grown under different nutrient treatments in a hydroponics experiment.

Plant nutrient	PC I	PC II	PC III
N	–0.462	0.508	0.005
P	0.351	–0.336	0.028
K	0.208	–0.008	0.638
Mg	0.332	–0.048	–0.235
Ca	0.287	–0.087	–0.406
S	0.321	0.046	0.460
B	0.311	–0.05	–0.437
Zn	0.307	0.128	–0.167
Mn	0.268	0.383	–0.133
Fe	–0.246	0.568	0.012
Cu	0.362	0.262	–0.092
Mo	0.310	–0.247	–0.190

correlation ($P = 0.02$) between PCI and greasy spot incidence. In PC II, loading factors for N, Mn, Fe, and Cu were positive and high, whereas loading factors for P and Mo were negative. The relationship between PC II and greasy spot incidence were negative ($P = 0.03$). Principal component III had high positive loadings for K and high negative loadings for Ca and B. The relationship between PC III and greasy spot incidence were negative ($P = 0.03$).

Canonical DFA was performed to determine the plant nutrients responsible for discriminating the seven treatments (Table 7). Discriminant function analysis determines the combination of variables to derive different orthogonal functions (canonicals). The first canonical explains the most variation, second explains the greatest part of the unexplained variation and so on. Therefore, the first canonical provides the most overall discrimination between groups. The DFA derives standardized beta coefficients for each independent variable in each canonical. The larger the

Table 7. Canonical structure loadings as determined by the canonical discriminant function analysis (DFA).

Variable	Canonical I	Canonical II
N	0.700	0.440
P	0.640	–1.827
K	0.420	–0.271
Mg	–3.792	0.320
Ca	–0.747	–0.584
S	–0.082	1.350
B	0.175	–0.176
Zn	–0.481	–0.940
Mn	–0.312	0.118
Fe	–0.918	0.066
Cu	–0.509	0.503
Mo	0.437	–0.388

standardized coefficient, the greater is the contribution of the respective variable to the group discrimination. Nitrogen, P, K, B, and Mo had high positive contribution to canonical I, whereas the contributions of Mg, Ca, Zn, Mn, Fe, and Cu were negative. In DFA, the individual scores of two canonicals are plotted and the resulting biplot shows the discrimination between groups. When the individual scores were plotted, three major clusters were found, indicating that the canonical I discriminated treatments T2 and T3 from each other and from rest of the treatments (Fig. 1). Canonical I had the highest contribution from Mg, hence separating Mg from other treatments and clustering T3 towards the lower values of canonical I. Treatment T2 was separated from other treatments due to its low N and clustered towards the negative values of canonical II (low N) and T3 was located towards the positive scores of canonical II. However, the other five treatments were located towards the positive scores of canonical I and II. Although clustered along with other treatments, T4 occurred towards the lower values of canonical I (low Ca) and T1 occurred towards the positive values of both canonical I and II.

Discussion

Our data show that greasy spot incidence in citrus was suppressed with the balanced mineral nutrition compared to the nutrient-deficient treatments in which one or more nutrients were omitted. In other words, plant health was improved with balanced mineral nutrition.

The nutritional status of a plant determines its histological or morphological structure and properties. Nutrients contribute to plant defense by altering metabolic pathways to enhance physiological resistance and by reducing pathogen inoculum and virulence (Huber, 1980). Specific nutrients may change the uptake and utilization of other nutrients, influence biological control, or enhance genetic resistance. Therefore, changes in nutrient concentration and composition of a fertilizer solution can significantly affect the disease occurrence. The degree of development of tomato bacterial canker (Corynebacterium michiganense Smith Jensen) increased with increasing concentration of Hoagland's nutrient solution including macro- and micronutrients from one tenth to twice that of the basal solution (Walker and Kendrick, 1948). In another study with tomato plants grown in a drip-sand culture, the unbalanced nutrient concentrations increased the development of verticillium wilt (Walker et al., 1954). Doubling the dose of N by fertigation compared with the conventional fertilization reduced the expression of deformation disease of *Gypsophila paniculata* mother plants (Ben-Yephet et al., 2006).

In our study, disease incidence and severity were high in Ca- and Mg-deficient treatments. Calcium suppresses diseases by increasing the structural integrity and resistance of the middle lamella and cell membranes to the extracellular enzymes of pathogens (Bateman and Basham, 1976). Therefore, plant sensitivity to many fungi increases in Ca-deficient plants due to ease of penetration into the cell wall. Magnesium acts as an enzyme cofactor and also helps in the stabilization of nucleotides and nucleic acids besides

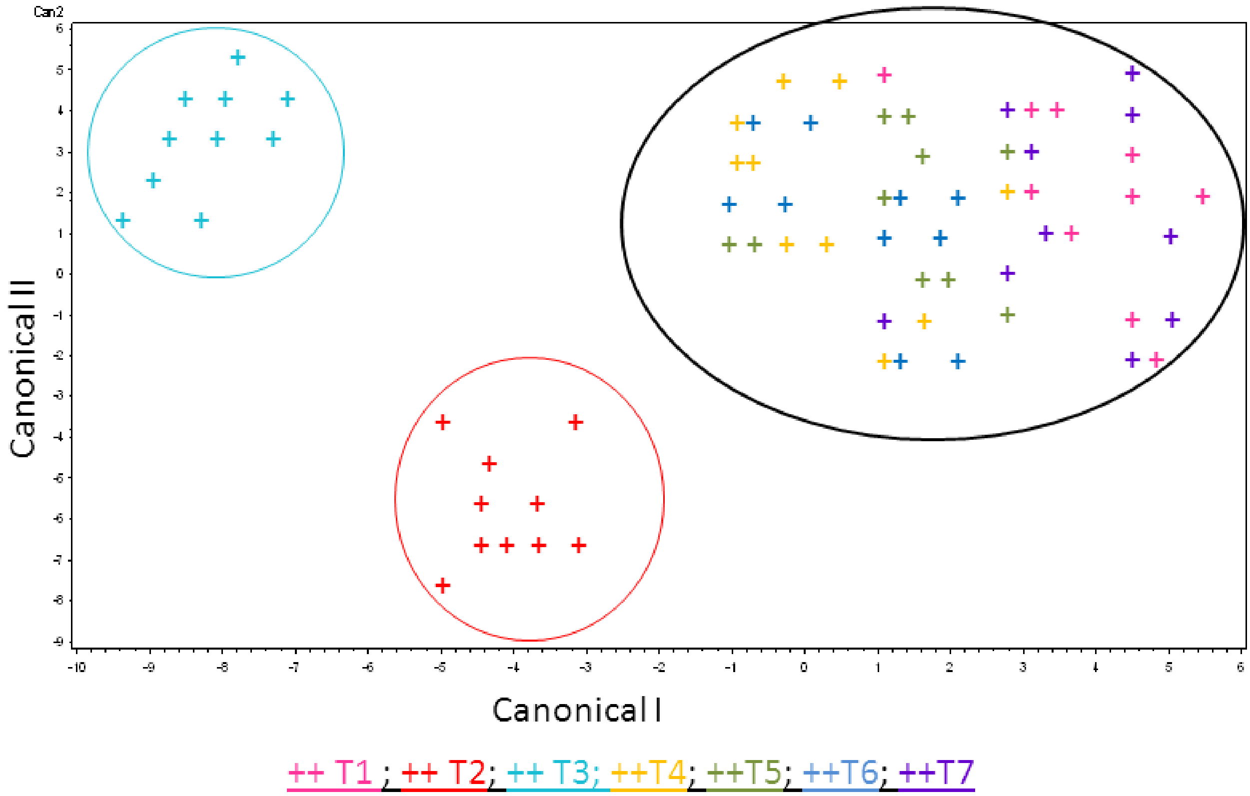

Fig. 1. Plot of canonicals for multiattributes and the group discrimination for greasy spot occurrence on 'Valencia' sweet orange trees grown under different nutrient treatments in a hydroponics experiment.

its central role in chlorophyll biosynthesis and function. It maintains the structural integrity of cell components and can reduce the susceptibility to extracellular enzymes produced by pathogens (Maathuis, 2009). Thus, both Ca and Mg contribute to the plant defense by providing a physical barrier against pathogen attack.

High disease incidence with the omission of B also supported the fact that unbalanced nutrition decreased plant disease resistance since leaf B content in this treatment was still in the optimum range for citrus trees. Boron plays a key role in the formation of carbohydrate-borate complexes, control of cell wall protein metabolism, cell membrane permeability and stability, and synthesis of phenolics and lignin (Stangoulis and Graham, 2007). Therefore, B deficient plants have a weaker physical barrier to pathogen infection and are more susceptible to plant diseases (Duffy et al., 1997; Graham and Webb, 1991).

High greasy spot incidence and severity in the Mn deficient treatment could be due to its diverse role in physiological functions of both plant growth and disease resistance.

Manganese plays a key role in photosynthesis and in the synthesis of lignin and phenols, aminopeptidase, and pectin methylesterase in controlling pathogen infection (Graham and Webb, 1991). Manganese has been shown to act as an essential component of plant resistance to a wide range of fungal and bacterial diseases such as powdery mildew, downy mildew, take-all, tan spot, and several others (Dordas, 2008; Huber and Graham 1999; Reuveni et al., 1997; Simoglou and Dordas, 2006).

We found that the electrolyte leakage was greater in nutrient-deficient treatments than in the full-strength treatment. The agreement between the disease incidence and electrolyte leakage suggests that the pathogen infection may have been responsible for leakage of electrolytes from cell membranes. The fungus penetrates the leaf surface (epidermal cells), either by passing between cells or through them. Therefore, the physical resistance presented by the strength and integrity of the cell walls and intercellular spaces is the plant's first line of defense. Electrolyte leakage represents cell membrane injury and has been correlated with the extent of damage due to various stresses including diseases (Balasubramanian, 1981; Shi et al., 2006; Tamuli et al., 2010). The balanced nutritional treatment prevented the electrolyte leakage, because the nutrients play a major role in the plant's ability to develop strong cell walls and tissue. For example, Ca, Mg, B, and Zn maintain the structural integrity and permeability of the cell membrane and prevent pathogen attack (Rice, 2007).

Besides electrolyte leakage, leaf sap pH can also be used as an indicator of plant health. Leaf sap pH was lower in the nutrient-deficient treatments (pH 6.0–6.2) than in the balanced nutrition treatment (pH 6.3). Also, the nutrient deficit treatments had high disease incidence and severity suggesting that the pH was lower due to the greasy spot pathogen infection. Plant pH relates to the acid/base balance and has been used to partition the healthy and diseased plants of various crops such as wheat, corn, sunflower, and tobacco in which the diseased plants were more acidic than the healthy plants (Harvey, 1920; Noyes and Hancock, 1981). Previous studies have documented the relationship between disease symptoms, plant tolerance, plant health, and plant sap pH (Noyes and Hancock, 1981; Robertson and Smith, 1931).

The position of a leaf on the plant also affected the electrolyte leakage and leaf sap pH. The lower leaves had the highest electrolyte leakage and lowest pH, which could be due to higher disease incidence on the lower leaves than on the middle leaves. The electrolyte leakage was also high in the upper leaves possibly due to those leaves being younger and more delicate.

Our plant nutrient analysis supported the fact that a lack of N is the limiting factor for citrus production (Reuther and Smith, 1954a). Results for plant nutrient analysis and disease incidence confirm Liebig's law of minimum (Leibig, 1855) and the need to provide plants with a correct balance of nutrients. Liebig recognized that any one deficiency could limit growth and leave other available nutrients unused or poorly utilized by the plant. In our study, reduced N levels decreased plant growth leading to the concentration of other nutrients in T2. The influence of nutrition on resistance and tolerance of host plants to pests and diseases is very complex. The deficiency of a particular nutrient may result in an increase of the other nutrients in the conductive tissue. Synergistic and antagonistic relationships between nutrients affect the uptake and utilization of other nutrients. For example, omitting K leads to the accumulation of P and nitrate-N, which further leads to increased bacterial infection; however, N is utilized by the plant when K is sufficient (Dordas, 2008; Maathuis, 2009). We observed that none of the plant nutrients was in the deficient or excess range in T1 (full-strength balanced nutrient solution) according to the guidelines for citrus tree nutrient analysis (Obreza et al., 2008). However, omitting any single nutrient from the balanced formula led to an imbalance of other nutrients and hence more disease occurrence. In the minus Ca treatment (T4), Ca was in the low range (1.5% to 2.9%), but not deficient (<1.5%); however, the low concentration of Ca still resulted in higher greasy spot incidence. The interaction of different nutrients with each other caused an imbalance of mineral nutrition. Potassium was in excess (>2.4%) and Zn was deficient ($< 18\ mg\cdot kg^{-1}$) in the treatment where Mg was omitted. This could be due to the high K uptake that triggers Zn uptake directly or by causing P-induced Zn deficiency (Cakmak and Marschner, 1986).

For PCA, the positive loading factors of all nutrients except N and Fe to PCI and positive correlation of PCI with greasy spot incidence suggests that low N and Fe contributed to high disease incidence and the opposite was true for other nutrients. This can be due to relatively higher nutrients in T2 that also had highest disease incidence. The positive loading factors of N, Mn, Fe, and Cu indicated that when these nutrients were low the disease incidence was high and vice-versa. The DFA showed that N, Mg, and Ca contributed significantly to the discrimination between different treatments and disease incidence.

Plant nutrient concentrations, PCA, and DFA revealed that omitting any one of the nutrients can be responsible for an excess or deficiency of another plant nutrient. Such unbalanced nutrition can bring an alteration of the susceptibility of plants to diseases. Since each nutrient functions as part of a delicately balanced interdependent system with the plant's genetics and the environment, it is important to establish a nutrient balance and appropriate nutrient proportions for optimum crop response. Low concentration of K or a high concentration of N may increase disease development as observed in fusarium wilt development in young tomato plants (Walker and Foster, 1946). Fusarium wilt of cotton, streptomyces scab of potato, clubroot of crucifers, and late blight of potato have been correlated with the ratio of K to Mg, Ca, and N, rather than the actual amount of K, Mg, Ca or N individually (Huber, 1980). Similarly, fusarium wilt of tomato, yellows of cabbage, stewart's wilt of corn, and downy mildew of tobacco are increased by K if there is an imbalance of other nutrient elements (Huber and Arny, 1985). Therefore, for protecting plants against a disease, not only is the supply of individual nutrients important, but balanced, crop specific nutrient ratios are also crucial for improving plant health (Huber, 1980).

To summarize, our results suggest that properly balanced nutrition is a critical factor in suppressing disease incidence. Our study provided the evidence that a small imbalance in the mineral nutrients can have adverse impacts on crop health and disease resistance. Therefore, balanced mineral nutrition may reduce pathogen invasion and use of pesticides, and enhance production efficiency.

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