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Assessment of Different Strategies to Balance High Mg Levels in the Irrigation Water when Preparing Nutrient Solution for Soilless Strawberry Crops

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Summary

In some cases, the levels of dissolved Mg in the irrigation water may exceed recommended Mg concentrations for hydroponic nutrient solutions (NS) to be used in strawberry (*Fragaria* × *ananassa* Duch.) crops. In this paper, three different strategies of managing a too high Mg concentration (2.1 mM) in irrigation water when preparing NS for hydroponically-grown strawberry crops, so as to minimize crop damage by excess Mg, were compared, using also a NS with a standard Mg level (1.4 mM) as control. In particular, the excessive Mg level was: i) either electrochemically balanced by equally increasing the sulphates, while not altering the levels of the other macrocations (Strategy I), ii) or accompanied by commensurate increases in the K, Ca and SO_4-S levels, thereby maintaining the same K:Ca:Mg ratio as in the control treatment (Strategy II), iii) or compensated for by proportional decreases in the K and Ca levels, so as to maintain the same total salt concentration as in the control treatment (Strategy III). The electrical conductivity (EC) in the control NS was 1.8 dS m^{-1} but increased to 1.9 and 2.6 dS m^{-1} by strategies I and II, respectively. When Strategy II was applied, the fresh shoot and fruit weights decreased to 78and 84 %, respectively, of those measured in the control treatment, despite the application of an optimal K:Ca:Mg ratio. These results indicate that, for strawberry performance, the EC of the supplied NS is more important than the K:Mg or Ca:Mg ratios. The reduction of plant growth and fruit yield by Strategy II was associated with an increase of EC to 3.4 dS m^{-1} in the drainage solution and significant reductions from −0.57 to −0.85 MPa in leaf water potential and from −1.1 to −1.4 MPa in leaf osmotic potential, while leaf turgor pressure and relative water content were similar in all treatments. Gas exchange parameters and chlorophyll levels were not affected by the increased Mg level, regardless of the strategy used to compensate for the excess Mg in the irrigation water.

Key words. *Fragaria* × *ananassa* – macrocations – soilless cultivation

Introduction

Soilless cultivation of strawberry has expanded considerably during the last decade in Mediterranean countries (Palencia et al. 2010). However, the primary irrigation water used to prepare nutrient solutions (NS) frequently contains salt ions such as Na^+, Cl^-, HCO_3^-, SO_4^{2-}, Ca^{2+} and/or Mg^{2+} at excessive concentrations (Sonneveld and Voogt 2009). The effects of NaCl salinity on soilless cultivated strawberry plants are well known (Awang et al. 1993; Saied et al. 2005). The presence of HCO_3^- in the irrigation water is adjusted by controlled addition of nitric acid, while the accumulation of SO_4^{2-} in the root environment is associated with salinity effects rather than specific ion toxicity (Savvas 2001; Sonneveld 2002) The responses of hydroponically-grown strawberry to different cation ratios when Ca exceeds recommended levels in the NS have been described in another paper (Neocleous and Savvas 2013). However, the impact of excessive Mg levels in the nutrient solution supplied to hydroponically-grown strawberry has been not investigated so far. Optimal plant growth and high fruit yields in soilless strawberry crops are achieved with relatively low concentrations of Mg and other macronutrients (De Kreij et al. 1999; Darnell and Stutte 2001; Tagliavini et al. 2005). Thus, the occurrence of Mg in the irrigation water at concentrations that are considered excessive for soilless strawberry plants is not a scarce problem, especially in Mediterranean countries. Consequently, too high Mg concentrations in the NS provided to hydroponic straw-

berry crops cannot be avoided. When the Mg concentration in the irrigation water, and concomitantly in the NS, exceeds the standard recommended level for soilless culture, growers may deploy one of the following three alternative strategies to minimize nutrient imbalances in the root zone. The first strategy is to maintain the concentrations of K and Ca to the recommended levels in the NS, thereby restricting the increase of the total ionic concentration, i.e. the electrical conductivity (EC), to that owing to the inevitably higher Mg concentration. The disadvantage of this strategy is the alteration of the recommended K:Ca:Mg ratio. The second strategy is to increase the K and Ca concentrations in the NS to levels maintaining the recommended K:Ca:Mg ratio. The disadvantage of this strategy is the increase of the EC in the NS that is supplied to the crop. The third strategy is to decrease the levels of K and Ca in the supplied NS to compensate for the increased Mg concentration, while not altering the standard K:Ca ratio, thereby maintaining the EC of the supplied NS to the recommended level. The disadvantage of this strategy is the decrease of the K and Ca levels in the supplied NS to levels below standard recommendations. As can be concluded from the above presentation of the three alternative strategies, all of them provide advantages and disadvantages, and thus it is not clear for the grower which one to apply in commercial soilless cultivations of strawberry.

In view of the above background, strawberry responses to the supply of NS with a high level of Mg while following one of the above outlined strategies with respect to the supply of the other two metallic cations (i.e. K and Ca) were investigated. To comparatively assess the responses of soilless strawberry crops to the three alternative strategies of balancing excess Mg in the irrigation water, plant growth, fruit yield, tissue nutrient concentrations, incidence of tipburn, gas exchange and chlorophyll parameters, water relations and fruit quality were measured.

Materials and Methods

The experiment was conducted in an unheated plastic-covered greenhouse at Zygi experimental farm of the Agricultural Research Institute, Cyprus (long. 33 °E, lat. 34 °N). Strawberry (*Fragaria* × *ananassa* Duch. cv. 'Camarosa') plants, developed in trays, were transplanted on 23 December 2011 into black plastic pots (20-cm in diameter, 20-cm height) containing 5 l of quartz sand. Four treatments differing in the composition of NS were arranged in a randomized block design with three replicates, each of them comprising 25 plants (one per pot). In each treatment, the NS was pumped from an independent tank and delivered to the top of each pot via drip emitters at a flow rate of 2 l h^{-1}. The irrigation was scheduled so as to achieve a target leaching fraction of 30 % (v/v) of the previous NS application, and the leachate was rejected. The NS treatments commenced on 28 December 2011 and the experiment was terminated on 27 April 2012. During the experiment, the temperature inside the greenhouse ranged from 12.7 to 20.6 °C and the relative humidity from 61.4 to 76.9 %.

Initially, the quartz sand was moistened to container capacity using the same starter NS (SNS; Table 1) in all treatments. The EC and pH in the above NS were 2.3 and 5.6 dS m^{-1}, respectively. Five days after planting, particularly on 28 December 2011, four different NS treatments (henceforth referenced as A, B, C, and D) were applied (Table 1). In particular, treatment A was a standard NS suitable for strawberry according to slightly modified suggestions of Sonneveld and Straver (1994), with a molar ratio of K:Ca:Mg = 0.55:0.31:0.14. Treatment B was a NS with increased Mg concentration (2.1 mM), the same K and Ca levels as in treatment A, and an EC value of 1.9 dS m^{-1}. Treatment C was a NS with increased Mg concentration (2.1 mM), which was accompanied by commensurate increases in K and Ca levels so as to maintain the same K:Ca:Mg ratio as in treatment A, and accordingly the EC value was raised to 2.6 dS m^{-1}. Treatment D was a NS with increased Mg concentration (2.1 mM), which was accompanied by commensurate decreases in K and Ca levels to maintain the same EC as in treatment A, and accordingly the K:Ca:Mg molar ratio was altered to 0.50:0.29:0.21. In treatments B and C, in which the concentrations of one or all major cations were altered with reference to treatment A, the changes in total cation charge were electrochemically balanced by equal variations in the SO_4^{2-} equivalent concentrations, whereas those of NO_3^- and $H_2PO_4^-$ were identical in all treatments. Desalinized water was used for the preparation of all NS. The micronutrient concentrations and the pH (5.6) were identical in all treatments (Table 1).

Fruit harvest started on 5 April 2012 (100 DAT) and lasted until 27 April 2012 (122 DAT). Fruits were har-

Table 1. Composition of the nutrient solutions used to moisten the substrate before planting (SNS)[a] and as experimental treatments A, B, C, and D[b].

Parameter	SNS	A	B	C	D
EC (dS m^{-1})*	2.30	1.80	1.90	2.60	1.80
K^+ (mM)	7.00	5.60	5.60	8.40	4.94
Ca^{2+} (mM)	6.00	3.20	3.20	4.80	2.83
Mg^{2+} (mM)	2.00	1.40	2.10	2.10	2.10
NO_3^- (mM)	17.00	12.00	12.00	12.00	12.00
SO_4^{2-} (mM)	2.50	1.23	1.93	4.93	1.23

[a] SNS contained $H_2PO_4^-$ = 1.3 mM; NH_4^+ = 1.00 mM; Fe, Mn, Zn, Cu, B, Mo = 30, 8, 6, 1, 30, 0.5 µM, respectively.
[b] A, B, C and D contained $H_2PO_4^-$ = 1.25 mM; NH_4^+ = 0.94 mM; Fe, Mn, Zn, Cu, B, Mo = 20, 10, 7, 0.8, 25, 0.5 µM, respectively.
* EC = electrical conductivity.

vested at a red stage, when considered commercially ripe according to standard cultural practices, and their fresh weight was recorded after removal of their calyxes. From each experimental unit, one fruit sample (40 fruits) was collected, then frozen, blended and kept at −30 °C for quality analysis. Leaf samples (most recent fully expanded functional leaves) and samples of NS and drainage solutions (DS) (n = 3) were collected 30 and 120 d after treatment initiation (DAT) and used to quantify mineral nutrients. Furthermore, 60 and 90 DAT, additional samples of DS were collected to monitor the nutrient status in the root zone.

Leaf, NS and DS samples were analyzed for the following mineral nutrients: N, P, K, Ca and Mg. Values of Ca and Mg in NS, DS and digested tissues (dry ashing at 580 °C for 16–18 h, dissolving the ash in 6 N HCl and assaying the solution obtained) were determined by atomic absorption spectrometry (Solaar M Series, Thermo Elemental, Cambridge, UK). Nitrogen concentrations in leaves were determined using the Kjeldahl procedure. Phosphorous determination was based on the formation of "molybdenum blue" using UV/Vis spectroscopy (Lambda 35, Perkin Elmer, MA, USA) and K was determined using flame photometry (Model 420, Sherwood Scientific, Cambridge, UK). The concentrations of NO_3^- and SO_4^{2-} in solution samples were measured using UV/Vis spectroscopy (APHA 2005).

Ten weeks after commencement of the treatments, the most recent, fully expanded leaves of six plants per replication were used in the early morning to determine gas exchange parameters, i.e. net photosynthetic rate (Pn), stomatal conductance (g_s), intercellular CO_2 concentration (Ci), and transpiration (E) using a Li-6400 (Li-Cor, Lincoln, NE, USA); water relations, i.e. leaf water potential (Ψ_w) using the pressure chamber SKPM 1400 (Skye Instruments Ltd, Llandrindod, UK), leaf osmotic potential (Ψ_π) using the microvoltometer HR-33T (Wescor Inc., Logan, UT, USA), leaf turgor pressure (Ψ_p) as $\Psi_w - \Psi_\pi$, and relative water content (RWC; Larcher 1980); chlorophyll parameters i.e. chlorophyll content (Lichtenthaler 1987) and chlorophyll fluorescence using OS-30p fluorometer (Opti-Sciences, Hudson, USA). Incidence of tipburn was estimated as described by Palencia et al. (2010).

Fruit quality i.e. soluble solids concentration (SS), pH, titratable acidity (TA), SS/TA ratio, dry matter content (DM), ascorbic acid (AA), total anthocyanins (TAn), total phenolics (TP) and antioxidant capacity (AC) of berry pulp, was also determined following the procedures described in a previous paper (Neocleous and Vasilakakis 2008). Plant growth, expressed in terms of fresh weight (FW) and dry weight (DW) of six complete plants, was measured after the experiment was terminated.

Measurements were performed on ten plants for each replicate, unless noted otherwise. Analysis of variance (ANOVA) of the data was performed using SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA). Treatment means were compared using Duncan's multiple range test (P = 0.05).

Results

Plants final fresh weight (FW) and dry weight (DW) (Fig. 1A), and fruit yield (Fig. 1B) were affected by NS treatments. Treatment C, i.e. the NS in which a high Mg level was combined with increased K and Ca levels and accordingly the total salt concentration (EC) rose, restricted both vegetative growth and fruit yield. Growth and fruit yield of the plants in treatments A, B and D were similar. The fruit quality parameters i.e., SS (avg. 7.26 °Brix); pH (avg. 3.67); TA (avg. 0.81 % citric acid); SS/TA ratio

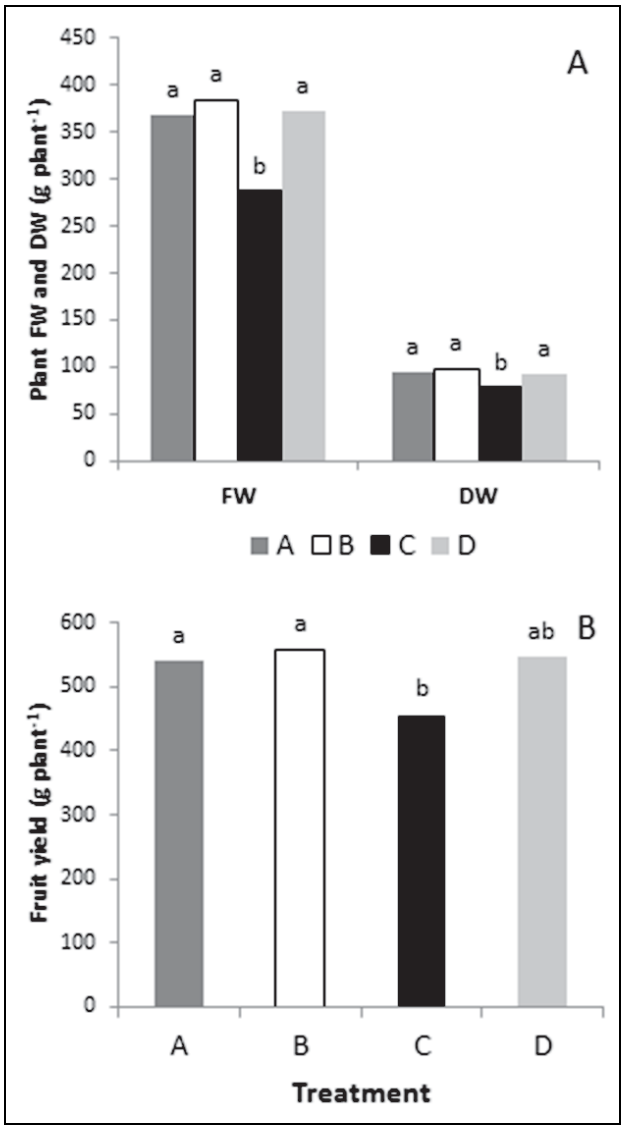


Fig. 1. Plant final fresh weight (FW) and dry weight (DW) (Fig. 1A) and fruit yield (Fig. 1B) in hydroponically-grown strawberry plants as influenced by the composition of the supplied nutrient solution (NS). Treatment A denotes a standard NS composition for strawberry, while treatments B, C, and D were obtained by applying different strategies to balance high Mg levels in the irrigation water when preparing NS for soilless strawberry crops. Bars with no common letter differ significantly (P = 0.05).

(avg. 8.99); DM (avg. 7.42 %); AA (avg. 33.1 mg AA [100 g FW]$^{-1}$); TAn (avg. 31.2 mg pelargonidin-3-glucoside [100 g FW]$^{-1}$]; TP (2.75 mg Gallic Acid [g FW]$^{-1}$); and AC (FRAP values; avg. 21.6 mol AA [g FW]$^{-1}$), did not reveal any differences imposed by the tested NS treatments.

The K concentration in the DS was significantly higher in treatment C than in the other three treatments throughout the experiment, reflecting the K differences in the supplied NS (Fig. 2A). The K level in DS of treatment A was higher than in DS from treatments B and D at the first sampling date, but these differences tended to disappear later on. The Ca concentrations in DS (Fig. 2B) exhibited similar differences with those found for K (Fig. 2A). The Mg concentration in DS was significantly lower in treatment A than in the other three treatments throughout the

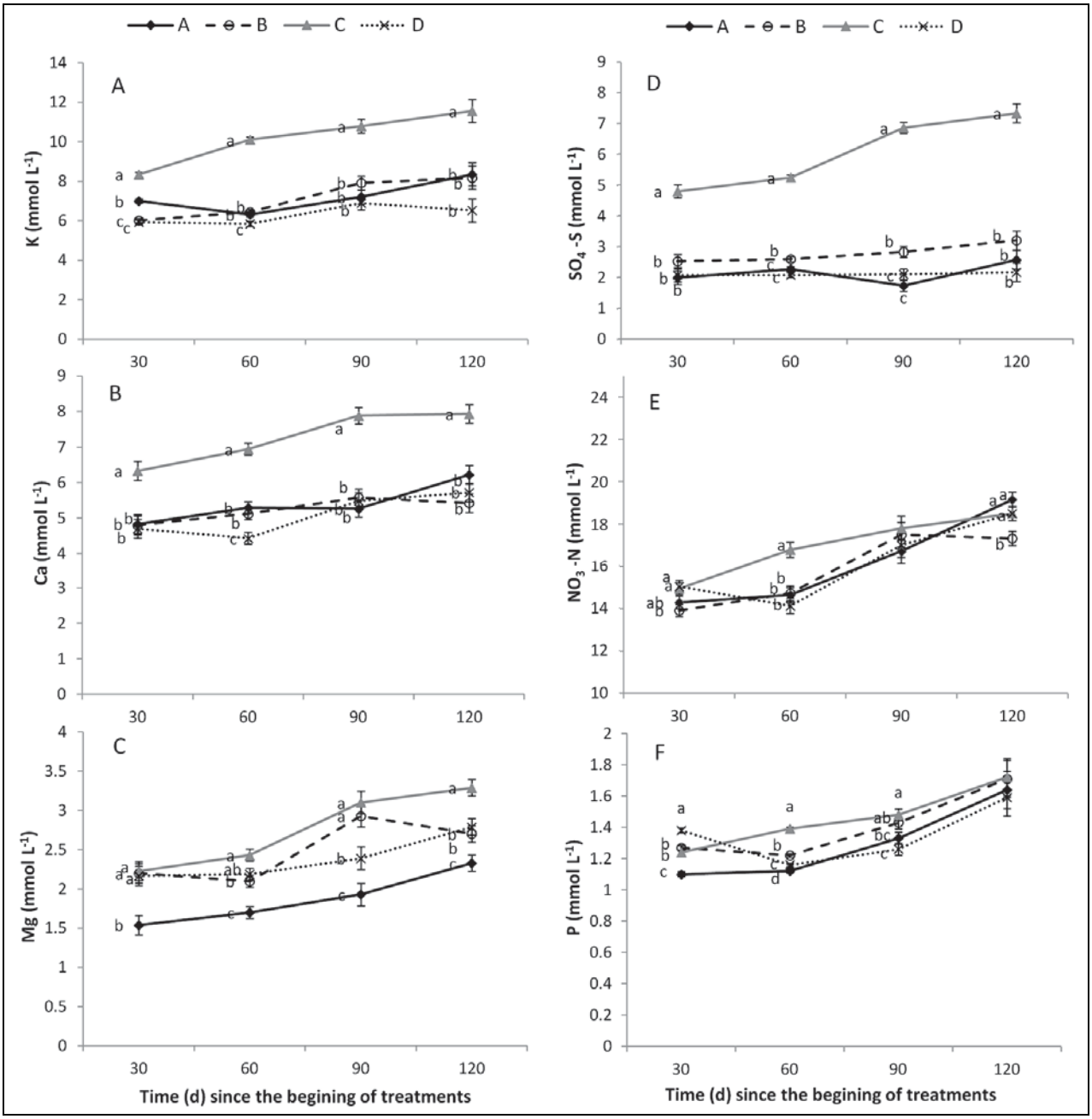


Fig. 2. Drainage solution composition (A–F) in hydroponically-grown strawberry plants as influenced by the composition of the supplied nutrient solution (NS). Treatment A denotes a standard NS composition for strawberry, while treatments B, C, and D were obtained by applying different strategies to balance high Mg levels in the irrigation water when preparing NS for soilless strawberry crops. Means with no common letter within each sampling date differ significantly (P = 0.05). Bars represent ± SE of means (n = 3).

experiment, reflecting the Mg differences in the supplied NS (Fig. 2C). However, treatment C showed a profound Mg accumulation in DS on the last sampling date although the Mg level in the supplied NS was the same as in treatments B, C and D (Table 1). The SO_4-S concentrations in DS (Fig. 2D) reflected the SO_4-S levels in the NS used (Table 1). The NO_3-N level in DS was affected by treatment (Fig. 2E) although the NO_3-N level in the supplied NS was identical in all treatments (Table 1). During the first and second sampling dates, the DS of treatment B exhibited similar NO_3-N concentrations with those found in DS of treatments A and D but lower levels compared to those of treatment C (Fig. 2E). These differences disappeared on the next sampling date, but on the last sampling treatment B exhibited a lower NO_3-N concentration in DS compared to the other three treatments (Fig. 2E). Finally, the P level in DS was affected by the tested NS treatments (Fig. 2F) although it was identical in the supplied NS (Table 1). On the first and second sampling dates, the P level in DS of treatment A had the lowest value compared with the other three treatments, which contained more Mg in the NS used. However, the differences disappeared on the last sampling date (Fig. 2F).

Throughout the experiment, the EC value in DS from treatment C was greater than that in DS from treatments A, B and D, while the EC value in the latter three treatments was similar (Fig. 3A). The EC in treatment C increased gradually up to 3.5 dS m^{-1} on the last sampling date, whereas the EC in the other three treatments increased up to 2.5 dS m^{-1}. On the first sampling date, the pH in DS of treatment A was significantly lower than that in DS from the other treatments in which a high Mg level (2.1 mM) was applied (Fig. 3B). However, these differences disappeared on the second sampling date and on the last sampling dates the pH in DS of treatment A showed the greatest value (Fig. 3B).

The concentrations of N, P and Mg in strawberry leaves were not affected by the tested macrocation treatments (Table 2). However, the strawberry plants in treatment C showed the highest leaf K level whereas the plants in treatment A showed the highest leaf Ca level. Despite these differences in leaf nutrient levels, the incidence of tipburn was not affected by the tested NS treatments (data not shown).

The strawberry plants in treatment C, i.e. the NS treatment with the highest EC, exhibited reductions in leaf water (Ψ_w) and leaf osmotic (Ψ_π) potential without alterations in leaf turgor pressure (Ψ_p) (Fig. 4) and relative water content (RWC) (avg. 86.3 %). The reduction of Ψ_w and Ψ_π was more profound in treatment C, followed by treatment B. Treatments A and D showed similar and less negative values. Evaluation of gas exchange and chlorophyll parameters did not indicate any differences arising from the treatments tested in the current study. In particular, net assimilation rate (Pn) averaged 17.5 μmol CO_2 m^{-2} s^{-1}; stomatal conductance (g_s), averaged 0.390 mol H_2O m^{-2} s^{-1}; intercellular CO_2 concentration (Ci) averaged 286 μmol CO_2 mol^{-1} and transpiration (E) averaged 2.93 mmol H_2O m^{-2} s^{-1}. Chlorophyll fluorescence parameter *Fv/Fm* ratio value (avg. 0.81) was in the optimal range for many plant species (Maxwell and Johnson 2000) and chlorophyll content averaged 1.31 mg g^{-1} FW.

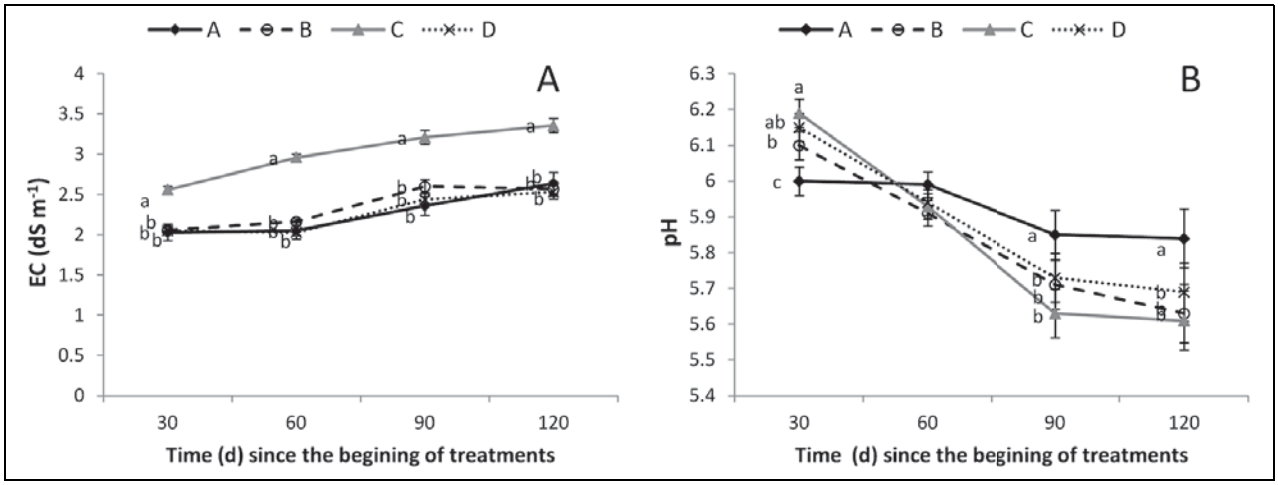


Fig. 3. Drainage solution EC (A) and pH (B) in hydroponically-grown strawberry plants as influenced by the composition of the supplied nutrient solution (NS). Treatment A denotes a standard NS composition for strawberry, while treatments B, C, and D were obtained by applying different strategies to balance high Mg levels in the irrigation water when preparing NS for soilless strawberry crops. Means with no common letter within each sampling time differ significantly (P = 0.05). Bars represent ± SE of means (n = 3).

Table 2. Macronutrient concentrations (% DW) in leaves of hydroponically-grown strawberry plants as influenced by the composition of the supplied nutrient solution (NS). Treatment A denotes a standard NS composition for strawberry, while treatments B, C, and D were obtained by applying different strategies to balance high Mg levels in the irrigation water when preparing NS for soilless strawberry crops.

Treatments	N	P	K	Ca	Mg
A	3.36a	0.84a	2.19b	0.80a	0.41a
B	3.38a	0.79a	2.18b	0.67b	0.42a
C	3.35a	0.78a	2.29a	0.72b	0.40a
D	3.32a	0.77a	2.11b	0.67b	0.44a

Mean values in each column that are followed by a different lower-case letter differ significantly (P = 0.05) according to the Duncan's multiple range test.

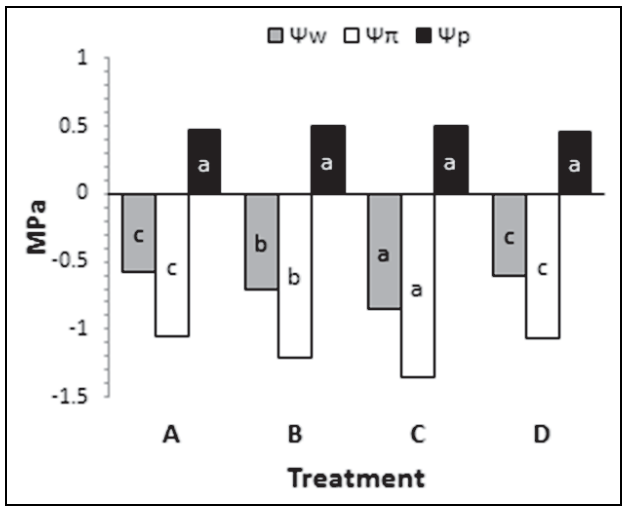


Fig. 4. Leaf water potential (Ψ_w), leaf osmotic potential (Ψ_π) and leaf turgor pressure (Ψ_p) in hydroponically-grown strawberry plants as influenced by the composition of the supplied nutrient solution (NS). Treatment A denotes a standard NS composition for strawberry, while treatments B, C, and D were obtained by applying different strategies to balance high Mg levels in the irrigation water when preparing NS for soilless strawberry crops. Bars with no common letter differ significantly (P = 0.05).

Discussion

The negative effects of high EC levels in the root zone owing to NaCl-salinity on strawberry fruit yield are well-known (Martinez-Barroso and Alvarez 1997; Saied et al. 2005; Orsini et al. 2012). As reported by Caruso et al. (2011), increasing NS strength resulted in a reduction of plant biomass and fruit yield despite the higher availability of nutrients of alpine strawberry grown in hydroponics. In line with the findings of Caruso et al. (2011), the profound yield decrease observed in treatment C, in which the EC in the DS increased to approximately 3.5 dS m^{-1} (Fig. 3A) due to accumulation of K, Ca, Mg, and SO_4^{2-} (Figs. 2A–D), revealed that strawberry is susceptible not only to NaCl but also to nutrient-induced salinity. Moreover, maintaining the target EC in the supplied NS by decreasing K and Ca to compensate for the excess Mg was more crucial for growth and yield than the failure to maintain the target K: Ca: Mg ratio or K, Ca and Mg concentrations in treatment D. These results reinforce previous findings of Neocleous and Savvas (2013) who reported that the damage caused by the increase of the EC when attempting to maintain a target K:Ca:Mg ratio despite excessive Ca levels in the irrigation water is higher than the benefits from the optimal cation ratio.

When comparing the leaf nutrient concentrations shown in Table 2 with the critical nutrient levels referenced for strawberry (Mills and Jones 1996; Almaliotis et al. 2002), it becomes clear that the reduced plant growth and fruit yield in treatment C cannot be ascribed to nutrient toxicities or deficiencies. Noteworthy, the leaf K was significantly higher in treatment C than in the other treatments, obviously as a result of the higher K level in the supplied NS (Adams 2002). Actually, the measured leaf K levels not only show that the concentration of this nutrient in plant leaves is influenced by the K level in the supplied solution (Willumsen et al. 1996; Adams 2002), but also reveals that strawberry plants are able to maintain K uptake rates at lower concentrations in the root environment (Sonneveld 2002). The significantly higher leaf Ca level in treatment A, compared with those measured in the three high-Mg treatments, was presumably a result of Ca and Mg completion in uptake. This finding reveals that Ca and Mg respond more sensitively to their mutual ratios in the root zone than to their absolute concentrations with respect to their uptake and transport to the leaves (Willumsen et al. 1996; Lieten 2006; San Bautista et al. 2009; Neocleous and Savvas 2013).

The change in water status imposed by a small increase in the external concentrations of soluble salts (EC) leads initially to growth suppression associated with osmotic effects (Munns 2002; Parida and Das 2005). The accumulation of macronutrients in the DS collected from treatment C fully justifies the profound effect of this treatment on water relations in the leaves of the strawberry plants. This effect is manifested in a stronger reduction of osmotic and water potential in leaves of strawberry plants in treatment C. These results are in line with those reported by Turhan et al. (2008) who found that 'Camarosa' strawberry plants are capable of osmotic adaptation when their roots are exposed to salinity. Although it is known that salt-stressed plants consume more metabolic energy to lower the osmotic potential in the leaves, thereby reducing the energy used for growth processes (Pasternak 1987), different acclimation mechanisms such us osmotic adjust-

ment, may lead to maintenance of net assimilation efficiency (Flexas et al. 1999). Indeed in the present study, the lower osmotic potential in treatment C was not associated with restricted photosynthetic parameters at stomatal or chloroplast level, indicating that photosynthetic apparatus was maintained intact and functioning, enabling plant to regulate metabolic processes. In this content, fruit quality parameters were not affected by the treatments applied in this study and corresponded well to earlier reports (Neocleous 2012), suggesting that adequate precursors were available in all cases.

Leaf osmotic potential (Ψ_π) and leaf water potential (Ψ_w) of strawberry plants became more negative in treatment C, whereas leaf turgor pressure (Ψ_p) (Fig. 4) and relative water content (RWC) (data not shown) were similar in all treatments. This finding may indicate that plants avoided a negative water balance through the greater decline in Ψ_π compared to Ψ_w, which led to turgor maintenance (Ψ_p) as suggested by Flexas et al. (2004) and Parida and Das (2005). However, the Ψ_w value that was observed in treatment C was very close to −1 MPa (Fig. 4), a level that has been considered as an early step of water stress (Hsiao 1973; Larcher 1980). As reported by Flexas et al. (2004), early stages of water stress may alter biochemical functions and, according to Munns (2002), the initial reduction of growth is probably due to hormonal signals generated by the roots. Thus, the reduction in plant growth and fruit yield, which was brought about by the increased external EC in treatment C, was presumably associated with metabolic limitations imposed by mild water stress in the root zone.

Conclusion

If the water used to prepare NS for hydroponic strawberry crops contains more Mg than 1.5 mM, which is considered as an optimal level for this plant species, increasing K and Ca levels in addition to that of Mg to maintain a standard K:Ca:Mg ratio is not suggested, because it raises the EC in the root zone. The EC-induced reductions of growth and fruit yield in strawberry plants seem to originate from a mild water stress and the concomitant adverse effects of water relations on metabolic processes. Concluding, when Mg exceeds recommended levels in the irrigation water used to prepare a NS, it is better to avoid any increase in the concentration of other macronutrient cations, thereby minimizing the EC increase in the supplied NS, because a too high EC in the root zone is more crucial than the K:Ca:Mg ratio for growth and fruit yield of hydroponically-grown strawberry plants.

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