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AND ENVIRONMENTAL CONTROL

Ph.D. Dissertation

Effect of greenhouse microclimate and water quality on nutrient uptake in pepper hydroponic crop

Andreas Ropokis, B.Sc., M.Ed.

Volos 2021

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Η έγκριση της Διδακτορικής Διατριβής από το Τμήμα Γεωπονίας Φυτικής Παραγωγής και Αγροτικού Περιβάλλοντος του Πανεπιστημίου Θεσσαλίας δε δηλώνει αποδοχή των γνωμών του συγγραφέα.

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ΠΕΡΙΛΗΨΗ

Η γλυκιά πιπεριά (*Capsicum annuum* L. ssp. *annuum*) είναι ένα λαχανικό θερμής εποχής, ευαίσθητο στην αλατότητα και ευρέως διαδεδομένο τόσο λόγω της οικονομικής του σημασίας όσο και λόγω της διατροφικής αξίας των καρπών του, οι οποίοι χαρακτηρίζονται από υψηλά επίπεδα αντιοξειδωτικών όπως το ασκορβικό οξύ, τα καροτενοειδή, το β-καροτένιο και φαινολικές ενώσεις. Σε καλλιέργειες πιπεριάς σε κλειστά υδροπονικά συστήματα ο καθαρός όγκος του παρεχόμενου νερού είναι ουσιαστικά ίσος με εκείνον που αφαιρείται μέσω της διαπνοής, εάν ανακυκλώνεται συνεχώς ολόκληρη η ποσότητα του διαλύματος απορροής. Επιπρόσθετα, ο λόγος εισροών μεταξύ της μάζας ενός θρεπτικού στοιχείου και του όγκου του νερού σε ένα κλειστό υδροπονικό σύστημα, είναι ίσος με τη συγκέντρωση αυτού του θρεπτικού στοιχείου στο θρεπτικό διάλυμα που παρέχεται στα φυτά για να αντισταθμιστεί η απορρόφηση θρεπτικών στοιχείων και νερού από τα φυτά. Το παραπάνω θρεπτικό διάλυμα το οποίο αναμειγνύεται με το διάλυμα απορροής που πρόκειται να ανακυκλωθεί ονομάζεται συνήθως "θρεπτικό διάλυμα για κλειστά συστήματα". Η μονάδα που χρησιμοποιείται για τη μέτρηση των αναλογιών απορρόφησης θρεπτικών στοιχείων προς νερό είναι η μάζα θρεπτικών στοιχείων ανά όγκο νερού, η οποία είναι ίδια με εκείνη που χρησιμοποιείται για τις συγκεντρώσεις σε θρεπτικά διαλύματα. Ως εκ τούτου, ο όρος "συγκέντρωση απορρόφησης" χρησιμοποιείται για να περιγράψει τις αναλογίες απορρόφησης θρεπτικών στοιχείων προς την απορρόφηση νερού.

Για να αποφευχθεί η εξάντληση ή η συσσώρευση θρεπτικών στοιχείων στη ζώνη της ρίζας σε μια καλλιέργεια που αναπτύσσεται σε ένα κλειστό υδροπονικό σύστημα, οι συγκεντρώσεις των στοιχείων στο θρεπτικό διάλυμα στα συστήματα αυτά θα πρέπει να είναι ίσες με τις αντίστοιχες αναλογίες απορρόφησης θρεπτικών στοιχείων προς νερού από τα φυτά. Η απορρόφηση θρεπτικών στοιχείων και η απορρόφηση νερού ανταποκρίνονται διαφορετικά στις αλλαγές στο μικρόκλιμα του θερμοκηπίου, καθώς αυτές είναι δύο πλήρως ανεξάρτητες φυσιολογικές διεργασίες. Ωστόσο, το διοξείδιο του άνθρακα και οι υδρατμοί μοιράζονται τις ίδιες οδούς μεταφοράς καθώς διέρχονται από τα στοματικά φύλλα, αν και σε διαφορετικές κατευθύνσεις. Ως αποτέλεσμα, οι διακυμάνσεις των αναλογιών διαπνοής προς απορρόφηση, όπως εκφράζονται σε όρους αποδοτικότητας χρήσης νερού, είναι μικρές για ένα συγκεκριμένο είδος φυτών. Δεδομένου ότι οι ρυθμοί σταθεροποίησης του άνθρακα καθορίζουν τόσο τη συσσώρευση της ξηρής μάζας των φυτών όσο και ταυτόχρονα τις απαιτήσεις των φυτών σε θρεπτικά στοιχεία, ο λόγος απορρόφησης θρεπτικού στοιχείου προς νερό παρουσιάζει αξιοσημείωτη σταθερότητα με το χρόνο όπως έχει αποδειχθεί από διάφορους

ερευνητές. Αυτό ισχύει κυρίως εάν οι κλιματικές συνθήκες δεν αλλάξουν δραματικά σε σχέση με τα βέλτιστα επίπεδα. Παρόλα αυτά, μεγάλες μεταβολές των κλιματολογικών παραμέτρων μπορούν να επηρεάσουν διαφορετικά τις αναλογίες απορρόφησης ενός ή περισσότερων θρεπτικών στοιχείων σε σύγκριση με τις αναλογίες απορρόφησης νερού, επιβάλλοντας έτσι ανάλογες αλλαγές στις αναλογίες απορρόφησής τους. Αυτό ισχύει ιδιαίτερα εάν μια κλιματική παράμετρος αποκλίνει έντονα από το βέλτιστο εύρος, επιβάλλοντας έτσι συνθήκες καταπόνησης που αλλάζουν με διαφορετικό τρόπο την απορρόφηση θρεπτικού στοιχείου από εκείνη του νερού, σε επίπεδο φυσιολογικό εκτός διαπνοής. Το κατώφλι θερμοκρασίας για την ανάπτυξη των περισσότερων ευαίσθητων στις χαμηλές θερμοκρασίες καρποδοτικών λαχανικών, όπως η πιπεριά, η μελιτζάνα, το αγγούρι, η τομάτα και το πεπόνι είναι περίπου 8-12°C.

Στα περισσότερα θερμοκήπια της Μεσογείου η θέρμανση κατά τη διάρκεια της ψυχρής περιόδου του έτους είτε δεν εφαρμόζεται είτε εφαρμόζεται σποραδικά, με στόχο απλώς τη διατήρηση της εσωτερικής θερμοκρασίας σε επίπεδα υψηλότερα από το ελάχιστο όριο ανάπτυξης. Ωστόσο, υπό συνθήκες χαμηλής θερμοκρασίας, ο μεταβολισμός των φυτών και οι συνακόλουθες απαιτήσεις σε θρεπτικά στοιχεία μπορεί να αλλάξουν σημαντικά σε σύγκριση με τις βέλτιστες συνθήκες θερμοκρασίας. Επιπλέον, οι ρυθμοί διαπνοής και ταυτόχρονα η απορρόφηση νερού μεταβάλλονται σημαντικά υπό συνθήκες χαμηλής θερμοκρασίας. Ως εκ τούτου, οι λόγοι απορρόφησης θρεπτικών στοιχείων προς νερό μπορεί να αλλάξουν σημαντικά σε σύγκριση με εκείνους που προσδιορίζονται υπό τις βέλτιστες συνθήκες θερμοκρασίας, εάν η μέση θερμοκρασία μέσα σε ένα θερμοκήπιο είναι σημαντικά χαμηλότερη από το βέλτιστο εύρος.

Οι τυποποιημένες συστάσεις για τα επίπεδα των μακρο- και μικροστοιχείων στο θρεπτικό διάλυμα κλειστών συστημάτων υδροπονικής καλλιέργειας πιπεριάς, βασίζονται κυρίως σε έρευνες που πραγματοποιήθηκαν στις Κάτω Χώρες. Ωστόσο, για ένα συγκεκριμένο είδος φυτού, οι τυποποιημένες συστάσεις σχετικά με τη σύνθεση ενός θρεπτικού διαλύματος σε κλειστά υδροπονικά συστήματα πρέπει να βασίζονται σε πειραματικά δεδομένα που προέρχονται από παρόμοιες κλιματικές ζώνες. Προηγούμενες έρευνες έδειξαν ότι στις υδροπονικές καλλιέργειες σε θερμές και ξηρές κλιματολογικές συνθήκες, όπως αυτές που επικρατούν στη λεκάνη της Μεσογείου, η συγκέντρωση απορρόφησης μπορεί να διαφέρει σημαντικά από εκείνη που παρατηρείται στα θερμοκήπια της Βόρειας Ευρώπης. Επιπλέον, στις συνθήκες αυτές περιορίζεται η διαθεσιμότητα νερού καλής ποιότητας λόγω χαμηλής βροχόπτωσης και υψηλής εξατμισοδιαπνοής και αυξάνονται οι ρυθμοί απελευθέρωσης ιόντων ασβεστίου στο εδαφικό διάλυμα. Ως αποτέλεσμα, η συγκέντρωση ασβεστίου στα ύδατα που χρησιμοποιούνται για την άρδευση κηπευτικών καλλιεργειών φθάνει σε υπερβολικά υψηλές τιμές σε σύγκριση με τις ανάγκες των φυτών. Λαμβάνοντας υπόψη ότι τόσο η τουριστική βιομηχανία όσο και η ακίνητη περιουσία στην ευρύτερη περιοχή της Μεσογείου αυξάνουν τις απαιτήσεις κατανάλωσης νερού, πολλοί καλλιεργητές έχουν ως μοναδική επιλογή την χρήση υπόγειων υδάτων με υπερβολικά υψηλές συγκεντρώσεις όχι μόνο χλωριούχου νατρίου, αλλά και ασβεστίου.

Τα συστήματα καλλιέργειας εκτός εδάφους σε θερμοκήπια μπορούν να συμβάλλουν όχι μόνο στη μείωση της κατανάλωσης του νερού λόγω ακριβέστερης δοσολογίας άρδευσης, αλλά επίσης και στην άρδευση με χρήση νερού οριακής ποιότητας σε σχέση με την αλατότητα, χωρίς προβλήματα για τα μέσα καλλιέργειας. Ωστόσο, στα συστήματα καλλιέργειας εκτός εδάφους, ένα σημαντικό κλάσμα θρεπτικού διαλύματος πρέπει να αποστραγγίζεται από τη ζώνη της ρίζας μετά από κάθε άρδευση, ώστε να εξασφαλίζεται η επαρκής παροχή νερού σε όλα τα φυτά. Το κλάσμα του αποστραγγιζόμενου νερού απορροής επηρεάζεται από διάφορες παραμέτρους, αλλά υπό φυσιολογικές συνθήκες ανάπτυξης πρέπει να κυμαίνεται μεταξύ 20% και 50%, αν και αυτή η τιμή μπορεί να αυξηθεί έως και 80%, όπως στην αρχή του κύκλου μιας καλλιέργειας ή σε χαμηλές θερμοκρασίες. Στα ανοικτά υδροπονικά συστήματα το νερό απορροής απορρίπτεται, αλλά αυτό οδηγεί σε σπατάλη πολύτιμων υδατικών πόρων καθώς και νιτροποίηση των υπόγειων υδάτων, γεγονός που οδηγεί σε αμφισβήτηση την περιβαλλοντική βιωσιμότητα των εκτός εδάφους ανοικτών συστημάτων καλλιέργειας. Προκειμένου να ελαχιστοποιηθούν οι περιβαλλοντικές επιπτώσεις της καλλιέργειας εντός του θερμοκηπίου θα μπορούσαν να εφαρμοστούν εκτός εδάφους κλειστά υδροπονικά συστήματα

Ένα συνηθισμένο πρόβλημα που ανακύπτει στα εκτός εδάφους κλειστά υδροπονικά συστήματα είναι η συσσώρευση ιόντων, συμπεριλαμβανομένων των μη θρεπτικών στοιχείων που μπορεί να είναι τοξικά και ως εκ τούτου ανεπιθύμητα ή θρεπτικών στοιχείων που περιέχονται σε υπερβολικές συγκεντρώσεις, ειδικά όταν το νερό της πηγής είναι χαμηλής ποιότητας. Στην τελευταία περίπτωση, οι λόγοι εισόδου ιόντων προς νερό (δηλαδή οι συγκεντρώσεις στο νερό άρδευσης) είναι υψηλότερες από τις αντίστοιχες αναλογίες απορρόφησης ιόντων προς νερό και αυτό οδηγεί στη συσσώρευση αυτών των θρεπτικών στοιχείων στη ζώνη της ρίζας. Σε μια καλλιέργεια πιπεριάς που αναπτύσσεται υπό αυτές τις ιδιαιτερότητες του νερού άρδευσης, οι συγκεντρώσεις απορρόφησης απαιτούν προσαρμογή με στόχο τη βελτιστοποίηση των θρεπτικών σχημάτων.

Στην παραγωγή λαχανικών, ο εμβολιασμός χρησιμοποιείται ως μια αποτελεσματική τεχνική φυτοπροστασίας για περισσότερα από 50 χρόνια και σε πολλά μέρη του κόσμου. Παρά το γεγονός ότι η εφαρμογή του στην εμπορική παραγωγή πιπεριάς είναι λιγότερο διαδεδομένη σε σύγκριση με την τομάτα, αγγούρι και καρπούζι, τα τελευταία χρόνια αρκετά υποκείμενα που χρησιμοποιήθηκαν για την εμβολιασμό πιπεριάς απέδειξαν ότι προσδίδουν σημαντική ανοχή σε παράγοντες βιοτικής και αβιοτικής καταπόνησης, αυξάνοντας το ενδιαφέρον μεταξύ των παραγωγών καλλιέργειας πιπεριάς θερμοκηπίου.

Έχοντας υπόψιν τα προαναφερόμενα, δημιουργήθηκαν τρεις πειραματικές εργασίες σε περιβάλλον θερμοκηπίου.

Στην πρώτη πειραματική εργασία, η έλλειψη δεδομένων στη διεθνή επιστημονική βιβλιογραφία σχετικά με την εκτίμηση των συγκεντρώσεων απορρόφησης σε πειράματα γλυκιάς πιπεριάς που καλλιεργούνται υπό θερμές και ξηρές κλιματολογικές συνθήκες, οδήγησε στον σχεδιασμό πειράματος με σκοπό τον υπολογισμό των μέσων τιμών συγκεντρώσεων απορρόφησης για τα περισσότερα μακρο- και μικροστοιχεία σε καλλιέργεια πιπεριάς που αναπτύχθηκε σε μεσογειακό περιβάλλον και συγκρισή των με αντίστοιχα δεδομένα που προέρχονται από βόρειο-Ευρωπαϊκές κλιματικές συνθήκες. Επιπλέον, δεδομένου της μεγάλης ποικιλίας εμπορικών τύπων πιπεριάς καθώς και της αυξανόμενης χρήσης εμβολιαζόμενων σπορόφυτων στην εγκατάσταση καλλιεργειών θερμοκηπίου πιπεριάς, εκτιμήθηκαν στο πείραμα αυτό οι συγκεντρώσεις απορρόφησης για τέσσερις διαφορετικού τύπου ποικιλίες πιπεριάς (*Capsicum annuum* L.), που αναπτύχθηκαν σε θάλαμο υαλόφρακτου θερμοκηπίου, εντός ανακυκλούμενου θρεπτικού διαλύματος και σύμφωνα με τις αρχές της καλλιέργειας σε λεπτή στοιβάδα θρεπτικού διαλύματος (Nutrient Film Technique- NFT). Οι δύο ποικιλίες που χρησιμοποιήθηκαν ήταν τύπου «Τετράλοβος» (Orangery, Sondela) και οι υπόλοιπες δύο ήταν τύπου «Φλωρίνης» (Bellisa) και «Κέρατο» (Sammy), από τις οποίες η ‘Sammy’ ήταν είτε αυτο-εμβολιαζόμενη είτε εμβολιαζόμενη στο εμπορικό υποκείμενο ‘RS10’ (*Capsicum annuum*). Οι τρεις μη εμβολιαζόμενες ποικιλίες και οι δύο συνδυασμοί της ‘Sammy’ δηλ. η αυτο-εμβολιαζόμενη και η εμβολιαζόμενη στο εμπορικό υποκείμενο ‘RS10’, αποτέλεσαν τις πέντε πειραματικές μεταχειρίσεις.

Το αρχικό θρεπτικό διάλυμα με το οποίο τροφοδοτήθηκαν τα φυτά είχε ηλεκτρική αγωγιμότητα (electrical conductivity -EC) 2,6 dS m^{-1} και περιείχε συγκεντρώσεις θρεπτικών στοιχείων: 6.00 mM K, 6.5 mM Ca, 2.0 mM Mg, 0.5 mM NH_4, 15.6 mM NO_3, 1.2 mM H_2PO_4, 6.5 mM SO_4^2, 15.0 μM Fe, 10.0 μM Mn, 7.0 μM Zn, 0.8 μM Cu, 50.0 μM B και 0.5 μM Mo. Μετά τη μεταφύτευση, τα θρεπτικά στοιχεία και το νερό που απορροφήθηκαν από τα φυτά αναπληρώνονταν καθημερινά με την παροχή θρεπτικού διαλύματος συμπλήρωσης με διαφορετικές συγκεντρώσεις θρεπτικών στοιχείων σε σχέση με το αρχικό θρεπτικό διάλυμα.

Κάθε τέσσερις εβδομάδες το ανακυκλούμενο θρεπτικό διάλυμα απομακρύνονταν και στην θέση του τοποθετούνταν φρέσκο θρεπτικό διάλυμα που περιείχε συγκεντρώσεις θρεπτικών στοιχείων: 6.0 mM K, 5.3 mM Ca, 1.65 mM Mg, 0.5 mM NH_4, 14.6 mM NO_3, 1.2 mM H_2PO_4, 2.0 mM SO_4, 20.0 μM Fe, 12.0 μM Mn, 6.0 μM Zn, 0.8 μM Cu, 45.0 μM B and 0.5 μM Mo. Το θρεπτικό διάλυμα που καταναλώθηκε από τα φυτά καταγράφονταν καθημερινά και αντικαταστάθηκε με τροφοδότηση θρεπτικού διαλύματος συμπλήρωσης με βάση το στάδιο ανάπτυξης των φυτών. Πιο συγκεκριμένα, για το βλαστικό στάδιο οι συγκεντρώσεις θρεπτικών στοιχείων ήταν: 5.3 mM K, 3.15 mM Ca, 1.3 mM Mg, 1.4 mM NH_4, 11.6 mM NO_3, 1.1 mM H_2PO_4, 1.2 mM SO_4, 15.0 μM Fe, 10.0 μM Mn, 4.0 μM Zn, 0.8 μM Cu, 30.0 μM B και 0.5 μM Mo. Οι αντίστοιχες συγκεντρώσεις για το αναπαραγωγικό στάδιο ήταν: 6.0 mM K, 2.7 mM Ca, 1.1 mM Mg, 0.80 mM NH_4, 10.6 mM NO_3, 1.1 mM H_2PO_4, 1.1 mM SO_4, 15.0 μM Fe, 10.0 μM Mn, 0.7 μM Cu, 5.0 μM Zn, 25.0 μM B και 0.5 μM Mo. Για την εκτίμηση των συγκεντρώσεων απορρόφησης χρησιμοποιήθηκε η μέθοδος υπολογισμού που βασίζεται στην απομάκρυνση των θρεπτικών στοιχείων και του νερού από το ανακυκλούμενο θρεπτικό διάλυμα.

Τα αποτελέσματα έδειξαν ότι η ‘Bellisa’ και η ‘Sondela’ είχαν αθροιστική κατανάλωση νερού περίπου 15% λιγότερο από την ‘Orangery’ και την αυτο-εμβολιαζόμενη ‘Sammy’ και περίπου 20% λιγότερο από την εμβολιαζόμενη ‘Sammy’ στο εμπορικό υποκείμενο RS10. Οι παραπάνω διαφορές στην αθροιστική κατανάλωση νερού δεν είχαν καμία σημαντική επίδραση στην συνολική παραγωγή καρπών, η οποία παραγωγή ήταν παρόμοια σε όλες τις ποικιλίες, χωρίς σημαντικές διαφορές μεταξύ τους. Ωστόσο ο αριθμός των καρπών ανά φυτό και το μέσο βάρος των καρπών επηρεάστηκαν σημαντικά από τον γονότυπο, με τις ‘Orangery’ και ‘Sondela’ να παρήγαγαν πολύ λιγότερους καρπούς από την ‘Sammy’, αλλά το βάρος των καρπών της τελευταίας ήταν επίσης πολύ μικρότερο. Ο εμβολιασμός της ‘Sammy’ στο εμπορικό υποκείμενο ‘RS10’ δεν είχε αντίκτυπο στη συνολική παραγωγή καρπών, ούτε στον αριθμό καρπών ανά φυτό ή στο μέσο βάρος καρπών και δεν βελτίωσε την αποδοτικότητα χρήσης νερού της ‘Sammy’. Η ‘Sondela’ λόγω της παρόμοιας απόδοσης και της σημαντικά χαμηλότερης αθροιστικής κατανάλωσης νερού σε σύγκριση με τις ‘Sammy’ και ‘Bellisa’, εμφάνισε σημαντικά υψηλότερη αποδοτικότητα χρήσης νερού από τις δύο αυτές ποικιλίες. Ωστόσο, η αποτελεσματικότητα χρήσης νερού της ‘Orangery’ ήταν τόσο υψηλή όσο αυτής της ‘Sondela’.

Ανεξάρτητα από την ποικιλία, η συγκέντρωση ασβεστίου στο ανακυκλούμενο θρεπτικό διάλυμα μειώθηκε αισθητά στο χρονικό διάστημα μεταξύ της έναρξης της άνθησης και της έναρξης της συγκομιδής, επειδή η συγκέντρωση απορρόφησης ασβεστίου μεγιστοποιήθηκε. Μετά την έναρξη της συγκομιδής, η συγκέντρωση απορρόφησης του ασβεστίου τείνει να μειώνεται σταδιακά κάτι που οδήγησε σε αυξημένα επίπεδα ασβεστίου στο ανακυκλούμενο θρεπτικό διάλυμα.

Η συγκέντρωση μαγνησίου στο ανακυκλούμενο θρεπτικό διάλυμα τείνει να μειώνεται ελαφρά μέχρι την έναρξη της συγκομιδής, ενώ στη συνέχεια έδειξε μια αυξητική τάση η οποία ήταν πολύ αδύναμη στη 'Sondela' και ισχυρότερη στη 'Sammy', ανεξάρτητη επίσης και από τον γονότυπο του υποκειμένου. Μετά την έναρξη της ανθοφορίας, οι συγκεντρώσεις απορρόφησης μαγνησίου παρέμειναν σχεδόν σταθερές καθόλη τη διάρκεια της περιόδου καλλιέργεια και ανεξάρτητα από την ποικιλία. Σε όλη τη διάρκεια της καλλιεργητικής περιόδου, οι υψηλότερες συγκεντρώσεις απορρόφησης μαγνησίου μεταξύ των ποικιλιών μετρήθηκαν στη 'Sondela'.

Η συγκέντρωση καλίου στο ανακυκλούμενο θρεπτικό διάλυμα αυξάνονταν ελαφρά μετά την έναρξη της ανθοφορίας και έντονα μετά την έναρξη της συγκομιδής και μέχρι 85 ημέρες μετά τη μεταφύτευση, με αντίστοιχη μείωση της συγκέντρωσης απορρόφησης καλίου. Στη συνέχεια, η συγκέντρωση απορρόφησης του καλίου αυξήθηκε ξανά σε παρόμοια επίπεδα με αυτά που μετρήθηκαν στο διάστημα μεταξύ της έναρξης της άνθησης και της συγκομιδής. Η 'Belissa' παρουσίασε σταθερά υψηλότερη συγκέντρωση απορρόφησης καλίου από τις άλλες ποικιλίες καθ' όλη τη διάρκεια της καλλιέργειας και αυτό οδήγησε σε χαμηλότερες συγκεντρώσεις καλίου στο ανακυκλούμενο θρεπτικό διάλυμα μετά την έναρξη της ανθοφορίας. Αντίθετα, η 'Orangery' παρουσίασε χαμηλότερη συγκέντρωση απορρόφησης καλίου μετά την έναρξη της συγκομιδής από τις άλλες ποικιλίες, με τις διαφορές ωστόσο να μην ήταν πάντα σημαντικές.

Οι συγκεντρώσεις ολικού αζώτου στο θρεπτικό διάλυμα ανακύκλωσης μειώθηκαν ελαφρά κατά την περίοδο συγκομιδής, ενώ παράλληλα η συγκέντρωση απορρόφησης ολικού αζώτου ακολούθησε μια αυξητική πορεία μέχρι την έναρξη σχεδόν της συγκομιδής και στην συνέχεια παρέμεινε έως το τέλος της συγκομιδής σταθερή. Οι αυτο-εμβολιαζόμενες 'Sammy' απορρόφησαν λιγότερο άζωτο ανά λίτρο νερού μετά την έναρξη της ανθοφορίας και στο πολύ πρώιμο στάδιο της συγκομιδής, αλλά στη συνέχεια η συγκέντρωση απορρόφησης ολικού αζώτου δεν επηρεάστηκε από την ποικιλία πιπεριάς.

Η συγκέντρωση φωσφόρου στα ανακυκλούμενο θρεπτικό διάλυμα έδειξε μια αυξανόμενη τάση στο διάστημα από την μεταφύτευση μέχρι την έναρξη της ανθοφορίας στις αυτο-εμβολιαζόμενες 'Sammy', 'Orangery' και 'Sondela', που συνοδεύτηκε από αντίστοιχη μείωση της συγκέντρωσης απορρόφησης φωσφόρου στο ίδιο χρονικό διάστημα. Αντίθετα, τα επίπεδα της συγκέντρωσης απορρόφησης φωσφόρου και συγκεντρώσης φωσφόρου στο ανακυκλούμενο θρεπτικό διάλυμα της 'Belissa' και της εμβολιαζόμενη 'Sammy' στο 'RS10', δεν άλλαξαν κατά το χρονικό διάστημα της έναρξης της άνθησης, σε σύγκριση με το αρχικό στάδιο βλαστικής ανάπτυξης. Μετά την έναρξη της ανθοφορίας, τόσο η συγκέντρωση φωσφόρου στο ανακυκλούμενο θρεπτικό διάλυμα όσο και η συγκέντρωση απορρόφησης φωσφόρου παρέμειναν σταθερές μέχρι το τέλος της καλλιέργειας.

Οι συγκεντρώσεις απορρόφησης μαγγανίου και ψευδαργύρου ήταν υψηλότερες κατά τη διάρκεια του σταδίου της βλαστικής ανάπτυξης και ακολούθως μειώνονταν ελαφρά αλλά συνεχώς έως μετά την έναρξη της συγκομιδής, ενώ δεν υπήρξαν σημαντικές διαφορές μεταξύ των διαφόρων ποικιλιών. Η συγκέντρωση απορρόφησης σιδήρου μειώνονταν επίσης σταθερά κατά τη διάρκεια της περιόδου καλλιέργειας, χωρίς σημαντικές διαφορές μεταξύ των εξεταζόμενων ποικιλιών. Η συγκέντρωση απορρόφησης βορίου μειώθηκε κατά τη διάρκεια του σταδίου βλαστικής ανάπτυξης έως και μετά την έναρξη της συγκομιδής και παρέμεινε περίπου σταθερή στην συνέχεια κατά τη διάρκεια του αναπαραγωγικού σταδίου. Κατά τη διάρκεια του αναπαραγωγικού σταδίου, δηλαδή μετά από τους τρεις πρώτους μήνες από τη μεταφύτευση, η συγκέντρωση απορρόφησης βορίου της ‘Sondela’ ήταν σημαντικά υψηλότερη από αυτής των άλλων εξεταζόμενων ποικιλιών. Επίσης, ο εμβολιασμός της ‘Sammy’ στο υποκείμενο RS10 δεν είχε σημαντική επίδραση στην συγκέντρωση απορρόφησης των μικροστοιχείων.

Συμπερασματικά, η παρούσα μελέτη έδειξε ότι το στάδιο ανάπτυξης των φυτών πιπεριάς έχει ισχυρό αντίκτυπο στις συγκεντρώσεις απορρόφησης των θρεπτικών στοιχείων. Για τα περισσότερα θρεπτικά μακρο-στοιχεία, δηλαδή κάλιο, ασβέστιο, μαγνήσιο και όλα τα υπό μελέτη μικροστοιχεία, οι συγκεντρώσεις απορρόφησης έτειναν να μειώνονταν με τον χρόνο και μόνο αυτές του ολικού αζώτου και φωσφόρου αυξάνονταν ελαφρά μετά το αρχικό βλαστικό στάδιο. Οι συγκεντρώσεις απορρόφησης των θρεπτικών στοιχείων είναι γνωστό ότι δεν αλλάζουν δραματικά κατά την περίοδο μιας καλλιέργειας. Στην παρούσα μελέτη, η τάση των συγκεντρώσεων απορρόφησης να μειώνονται με το χρόνο για τα περισσότερα θρεπτικά στοιχεία (εκτός του αζώτου και φωσφόρου) φαίνεται να οφείλεται κυρίως σε μια ισχυρότερη αύξηση του ρυθμού απορρόφησης του νερού στον χρόνο σε σχέση με τους ρυθμούς απορρόφησης θρεπτικών στοιχείων, αν και σε απόλυτες τιμές αυξάνεται η ζήτηση θρεπτικών στοιχείων.

Οι εξαιρέσεις των συγκεντρώσεων απορρόφησης αζώτου και φωσφόρου οι οποίες αυξήθηκαν ελαφρώς καθώς τα φυτά εισήλθαν στην αναπαραγωγική φάση, μπορεί να υποδεικνύουν μια ισχυρότερη αύξηση της ζήτησης σε άζωτο και φώσφορο κατά τη διάρκεια του αναπαραγωγικού σταδίου από ότι της αύξησης της ολικής φυτικής διαπνοής. Η αύξηση της ζήτησης φωσφόρου καθώς τα φυτά περνούν από το βλαστικό στο αναπαραγωγικό στάδιο μπορεί να αποδοθεί στις αισθητά υψηλότερες συγκεντρώσεις φωσφόρου στον καρπό πιπεριάς από ότι στα φύλλα. Η ελαφρά αύξηση της συγκέντρωσης απορρόφησης αζώτου όταν τα φυτά εισήλθαν στο στάδιο αναπαραγωγής στο συγκεκριμένο πείραμα, δεν μπορεί να αποδοθεί στις αυξημένες συγκεντρώσεις αζώτου στους καρπούς σε σύγκριση με εκείνες των φύλλων, καθώς οι συγκεντρώσεις αζώτου τείνουν να είναι υψηλότερες στα φύλλα από ότι στους καρπούς. Μια εναλλακτική ερμηνεία για αυτό είναι να υπάρχει μια πιθανή αύξηση των ποσοστών απονιτροποίησης με την γήρανση της καλλιέργειας, αύξηση που μπορεί να αποδοθεί στη σταδιακή αύξηση της θερμοκρασίας του αέρα, δεδομένου ότι η καλλιέργεια ξεκίνησε στα μέσα Ιανουαρίου και η έναρξη του σταδίου αναπαραγωγής συνέπεσε με την έλευση της άνοιξης. Οι απώλειες αζώτου μέσω απονιτροποίησης σε υδροπονικά θρεπτικά διαλύματα είναι γνωστό ότι μπορεί να είναι σημαντικές φτάνοντας σε επίπεδα έως και 20% της συνολικής προσφοράς. Σύμφωνα με τη μέθοδο που εφαρμόστηκε για την εκτίμηση της συγκέντρωσης απορρόφησης στο συγκεκριμένο πείραμα, οι απώλειες απονιτροποίησης λαμβάνονταν ως εμφανή απορρόφηση αζώτου και συνεπώς έτειναν να αυξάνουν την εκτιμώμενη συγκέντρωση απορρόφησης.

Οι σημαντικά υψηλότερες συγκεντρώσεις απορρόφησης ασβεστίου και μαγνησίου στη ‘Sondela’ και καλίου στη ‘Bellisa’ σε σύγκριση με εκείνες που καταγράφηκαν σε όλους τους άλλους γονότυπους οι οποίες ήταν σταθερές καθ 'όλη την περίοδο καλλιέργειας, οφείλονταν στον συνδυασμό τόσο των διαφορών στην ολική φυτική διαπνοή, εξαιτίας των διαφορών στην φυλλική επιφάνεια όσο και στη ζήτηση θρεπτικών στοιχείων. Πράγματι, η ‘Sondela’ και η ‘Bellisa’ είναι ποικιλίες που καλλιεργούνται για την παραγωγή καρπών κόκκινης πιπεριάς οι οποίες χρειάζονται περισσότερο χρόνο για να ωριμάσουν από εκείνες που συλλέγονται πράσινες, όπως συμβαίνει με την ‘Sammy’. Όμως είναι γνωστό ότι η καθυστέρηση στην συγκομιδή καρπών, αυξάνει μεν το φορτίο καρπών, αλλά καθυστερεί την βλαστική ανάπτυξη, μειώνοντας έτσι την φυλλική επιφάνεια ανά φυτό και ταυτόχρονα την ολική φυτική διαπνοή. Επιπλέον, ο παράγοντας που τελικά οδήγησε σε υψηλότερες συγκεντρώσεις απορρόφησης ασβεστίου και μαγνησίου στη ‘Sondela’ από ότι στους άλλους γονότυπους, ήταν η υψηλότερες συγκεντρώσεις ασβεστίου στα φύλλα και του μαγνησίου στα φύλλα και στους καρπούς. Η σημαντικά υψηλότερη συγκέντρωση απορρόφησης καλίου στη ‘Bellisa’ οφειλόταν επίσης στο συνδυασμό υψηλότερης ολικής φυτικής διαπνοής και υψηλότερων συγκεντρώσεων καλίου στα φύλλα. Οι συγκεντρώσεις απορρόφησης ολικού αζώτου και φωσφόρου δεν φαίνεται να επηρεάζονται από τον γονότυπο της ποικιλίας, ενώ εκείνες των σιδήρου, μαγγανίου, ψευδαργύρου και βορίου δεν έδειξαν σταθερή επίδραση του γονότυπου, αν και η ‘Sondela’ παρουσίασε σημαντικά υψηλότερες συγκεντρώσεις απορρόφησης ψευδαργύρου και βορίου από τις άλλες ποικιλίες σε ορισμένα στάδια ανάπτυξης. Η ‘Sondela’ εμφάνισε υψηλότερη συγκέντρωση απορρόφησης βορίου μόνο κατά τη διάρκεια του αναπαραγωγικού σταδίου ανάπτυξης, γεγονός που δείχνει την επίδραση του αριθμού καρπών στην απορρόφηση βορίου και λιγότερο επίδραση που σχετίζεται με τη διαπνοή. Το υποκείμενο πιπεριάς ‘RS10’ το οποίο χρησιμοποιήθηκε για τον εμβολιασμό της ‘Sammy’ ανήκει στο ίδιο είδος με το εμβόλιο (*Capsicum annuum*) και όχι σε άγριο συγγενικό είδος πιπεριάς που χαρακτηρίζεται από έντονο ριζικό σύστημα. Αυτός είναι πιθανότατα ο λόγος για την έλλειψη διαφορών στα επίπεδα θρεπτικών στοιχείων των φύλλων και καρπών καθώς και στις συγκεντρώσεις απορρόφησης μεταξύ φυτών ‘Sammy’ αυτο-εμβολιαζόμενων και φυτών ‘Sammy’ εμβολιασμένων στο υποκείμενο ‘RS10’. Οι συγκεντρώσεις απορρόφησης που βρέθηκαν σε αυτή τη μελέτη κυμαίνονταν από 2,4 - 3,7 mmol L^{-1} για ασβέστιο, 1,0 - 1,5 mmol L^{-1} για μαγνήσιο, 6,1 – 9,0 mmol L^{-1} για κάλιο, 11,7 - 13,7 mmol L^{-1} για άζωτο και 0,7 - 1,1 mmol L^{-1} για φώσφορο.

Στην δεύτερη πειραματική εργασία, για να ελεγχθεί το κατά πόσο η υποβέλτιστη ή χαμηλή θερμοκρασία και ο εμβολιασμός μεταβάλλουν τις συγκεντρώσεις απορρόφησης σε καλλιέργειες πιπεριάς που αναπτύσσονται σε μη θερμαινόμενα ή ανεπαρκώς θερμαινόμενα θερμοκήπια στις κλιματικές συνθήκες της Μεσογείου, σχεδιάστηκε ένα πείραμα σε δύο θαλάμους ενός θερμαινόμενου θερμοκηπίου όπου η ελάχιστη θερμοκρασία ημέρας/νύχτας διατηρήθηκε συνεχώς στους 21/16°C και στους 12/7°C στους θαλάμους 1 και 2, αντίστοιχα, κατά τη διάρκεια ολόκληρης της πειραματικής περιόδου. Οι παραπάνω θερμοκρασίες αντιστοιχούσαν σε μια τυπική και χαμηλής θερμοκρασίας καλλιεργητική μεταχείριση, αντίστοιχα. Στους παραπάνω θαλάμους, δύο ποικιλίες γλυκιάς πιπεριάς (*Capsicum annuum* L.) είτε αυτο-εμβολιαζόμενες είτε εμβολιαζόμενες σε δύο διαφορετικά εμπορικά υποκείμενα, αναπτύχθηκαν σε ανακυκλούμενο θρεπτικό διάλυμα σύμφωνα με τις αρχές της καλλιέργειας σε λεπτή στοιβάδα θρεπτικού διαλύματος (Nutrient Film Technique- NFT). Οι δύο ποικιλίες πιπεριάς ήταν η πρώτη τύπου «Τετράλοβος» (Orangery) και η δεύτερη επιμήκης τύπου «Κέρατο» (Sammy), ενώ τα δύο υποκείμενα ήταν το ‘Robusto’ και το ‘Terrano’, τα οποία ανήκουν στο *Capsicum annuum*. Οι έξι συνδυασμοί εμβολιασμού που μελετήθηκαν ήταν: ‘Sammy’/‘Sammy’, ‘Robusto’/‘Sammy’, ‘Terrano’/‘Sammy’, ‘Orangery’/‘Orangery’, ‘Robusto’/‘Orangery’ και ‘Terrano’/‘Orangery’. Κάθε συνδυασμός επαναλήφθηκε τρεις φορές και συνολικά χρησιμοποιήθηκαν 36 πειραματικά κλειστά υδροπονικά συστήματα κυκλοφορίας και για τους δυο θαλάμους.

Στις 29 Νοεμβρίου, εννέα φυτά από τους παραπάνω συνδυασμούς μεταφυτεύθηκαν στα 36 κλειστά υδροπονικά συστήματα κυκλοφορίας, δημιουργώντας πυκνότητα φυτών 2,5 φυτά ανά m^2 για κάθε θάλαμο. Κάθε κλειστό υδροπονικό σύστημα κυκλοφορίας περιλάμβανε ένα κανάλι, ατομική δεξαμενή τροφοδοσίας, αντλία και σωλήνες άρδευσης. Πριν από τη μεταφύτευση, σε όλες τις δεξαμενές τροφοδοσίας τοποθετήθηκε αρχικό θρεπτικό διάλυμα. Η στάθμη του θρεπτικού διαλύματος στην δεξαμενή τροφοδοσίας διατηρούνταν σταθερή, χρησιμοποιώντας έναν φλοτέρ και έναν σωλήνα συνδεδεμένο με μια δεξαμενή συμπλήρωσης, που ήταν τοποθετημένη πάνω από την δεξαμενή τροφοδοσίας. Το αρχικό θρεπτικό διάλυμα που καταναλώνονταν από τα φυτά στην δεξαμενή τροφοδοσίας, αντισταθμίζονταν με αυτόματη έγχυση ενός θρεπτικού διαλύματος συμπλήρωσης κατάλληλης σύνθεσης για καλλιέργεια πιπεριάς σε κλειστά υδροπονικά συστήματα, το οποίο περιέχονταν στη δεξαμενή συμπλήρωσης. Η σύνθεση του θρεπτικού διαλύματος συμπλήρωσης ήταν περίπου ίδια με τις τυπικές συγκεντρώσεις απορρόφησης για καλλιέργεια πιπεριάς που εκτιμήθηκαν στο προηγούμενο πείραμα. Αμέσως μετά τη μεταφύτευση συλλέχθηκαν δείγματα αρχικού διαλύματος και διαλύματος συμπλήρωσης από όλα τα κλειστά υδροπονικά συστήματα κυκλοφορίας, ενώ η τελευταία συλλογή αντίστοιχων δειγμάτων συλλέχθηκε μετά την ολοκλήρωση 790 βαθμοημερών και στους δύο θαλάμους. Λόγω των διαφορετικών συνθηκών θερμοκρασίας σε κάθε θάλαμο, ο χρόνος που απαιτείται για την επίτευξη των ίδιων βαθμοημερών ήταν διαφορετικός, με την έναρξη της συγκομιδή στους θαλάμους 1 και 2 να ήταν στις 6 Φεβρουαρίου και 23 Μαρτίου αντίστοιχα και το τέλος της συγκομιδής στις 5 Μαΐου για τον θάλαμο 1 και 20 Μαΐου για τον θάλαμο 2. Οι μέσες αναλογίες απορρόφησης θρεπτικών στοιχείων προς νερό (συγκεντρώσεις απορρόφησης) για το ασβέστιο, μαγνήσιο, κάλιο, ολικό άζωτο, φώσφορο, σίδηρο, μαγγάνιο, ψευδάργυρο και βόριο υπολογίστηκαν μετρώντας την ολική απορρόφηση κάθε θρεπτικού στοιχείου και της συγκέντρωσης απορρόφηση νερού από το ανακυκλούμενο θρεπτικό διάλυμα κατά τη διάρκεια καλλιεργητικής περιόδου που αντιστοιχούσε σε 790 βαθμοημέρες και για τους δύο θαλάμους.

Η καλλιεργητική μεταχείριση με χαμηλές θερμοκρασίες του θαλάμου 2 μείωσε τη συνολική παραγωγή καρπών κατά περίπου 50% στη ‘Sammy’ και στο 33% στην ‘Orangery’, ανεξάρτητα από τους συνδυασμούς εμβολιασμού. Ο εμβολιασμός στο υποκείμενο ‘Robusto’ δεν είχε αντίκτυπο στην κατανάλωση νερού των φυτών, ενώ αντίθετα ο εμβολιασμός στο ‘Terrano’ αύξησε την κατανάλωση νερού των φυτών μόνο όταν το εμβόλιο ήταν η ‘Orangery’. Η αποδοτικότητα χρήσης νερού μειώθηκε σημαντικά στις χαμηλές θερμοκρασίες του θαλάμου 2, ανεξάρτητα από το συνδυασμούς υποκειμένου/εμβολίου. Η κατανάλωση νερού των φυτών αυξήθηκε σημαντικά στις χαμηλές θερμοκρασίες του θαλάμου 2 με τον εμβολιασμό της ‘Sammy’ στα υποκείμενα ‘Robusto’ και ‘Terrano’, ενώ στις τυπικές θερμοκρασίες του θαλάμου 1 αυτό συνέβη μόνο όταν το υποκείμενο της ‘Sammy’ ήταν το ‘Robusto’. Ωστόσο, ο εμβολιασμός στην ‘Orangery’ δεν είχε καμία επίδραση στην κατανάλωση νερού των φυτών, ανεξάρτητα από το υποκείμενο και τις θερμοκρασίες των θαλάμων.

Η συγκέντρωση απορρόφησης καλίου ήταν σημαντικά υψηλότερη στην μεταχείριση με τις χαμηλές θερμοκρασίες του θαλάμου 2 σε σύγκριση με τις τυπικές θερμοκρασίες του θαλάμου 1, τόσο στα αυτο-εμβολιαζόμενα φυτά όσο και στα φυτά εμβολιαζόμενα στο 'Robusto', ανεξάρτητα από το εμβόλιο. Ωστόσο, οι διαφορές αυτές δεν ήταν σημαντικές στα φυτά των 'Sammy' και 'Orangery' που είχαν εμβολιασθεί στο 'Terrano', επειδή το τελευταίο αύξησε σημαντικά τη συγκέντρωση απορρόφησης καλίου τόσο στην 'Orangery' όσο και στην 'Sammy' στις τυπικές θερμοκρασίες σε σύγκριση με τους άλλους συνδυασμούς εμβολιασμού.

Η συγκέντρωση απορρόφησης ασβεστίου δεν επηρεάστηκε από κανένα συνδυασμό εμβολιασμού, αλλά ήταν σημαντικά υψηλότερη στις χαμηλές θερμοκρασίες του θαλάμου 2 του θερμοκηπίου. Η συγκέντρωση απορρόφησης του μαγνησίου, όπως και του καλίου, ήταν σημαντικά υψηλότερη στην μεταχείριση με τις χαμηλές θερμοκρασίες του θαλάμου 2 σε σύγκριση με τις τυπικές θερμοκρασίες του θαλάμου 1, τόσο στα αυτο-εμβολιαζόμενα φυτά όσο και στα φυτά εμβολιαζόμενα στο 'Robusto', ανεξάρτητα από το εμβόλιο, ενώ δεν επηρεάστηκε από τις χαμηλές θερμοκρασίες του θαλάμου 2 όταν αμφότερες οι ποικιλίες είχαν εμβολιαστεί στο 'Terrano'. Ο εμβολιασμός τόσο στο 'Robusto' όσο και στο 'Terrano' αύξησε την συγκέντρωση απορρόφησης μαγνησίου στις τυπικές θερμοκρασίες, ενώ στις χαμηλές θερμοκρασίες μόνο το 'Robusto' αύξησε συγκέντρωση απορρόφησης μαγνησίου.

Η συγκέντρωση απορρόφησης ολικού αζώτου ήταν σημαντικά χαμηλότερη, ενώ αυτήν του φωσφόρου ήταν σημαντικά υψηλότερη, στις τυπικές θερμοκρασίες του θαλάμου 1 σε σύγκριση με τη χαμηλές θερμοκρασίες του θαλάμου 2 και αυτό ανεξάρτητα από την ποικιλία και τον γονότυπο του υποκειμένου. Οι συνδυασμοί υποκειμένων/εμβολίων δεν είχαν καμία σημαντική επίδραση στις συγκεντρώσεις απορρόφησης ολικού αζώτου και φωσφόρου, ενώ και η αλληλεπίδραση μεταξύ των συνθηκών θερμοκρασίας και του εμβολιασμού ήταν μη σημαντική.

Υπό συνθήκες τυπικών θερμοκρασιών του θαλάμου 1 οι συγκεντρώσεις απορρόφησης των σιδήρου και ψευδαργύρου ήταν σημαντικά υψηλότερες ενώ η αντίστοιχη του μαγγανίου ήταν σημαντικά χαμηλότερες, σε σύγκριση με αυτές των χαμηλών θερμοκρασιών του θαλάμου 2, χωρίς καμία σημαντική αλληλεπίδραση μεταξύ των συνθηκών θερμοκρασίας και του συνδυασμών υποκειμένων/εμβολίων. Επιπλέον, οι συνδυασμοί εμβολιασμού δεν είχαν σημαντική επίδραση στις συγκεντρώσεις απορρόφησης των σιδήρου και ψευδαργύρου. Ωστόσο, η συγκέντρωση απορρόφησης μαγγανίου επηρεάστηκε σημαντικά τόσο από την ποικιλία όσο και από τον εμβολιασμό. Συγκεκριμένα, η ‘Orangery’ απορρόφησε σημαντικά περισσότερο μαγγάνιο ανά λίτρο απορροφούμενου νερού από τη ‘Sammy’, ανεξάρτητα του γονότυπου του υποκειμένου. Επιπλέον, ο εμβολιασμός της ‘Orangery’ τόσο στο ‘Robusto’ όσο και στο ‘Terrano’ μείωσε τη μάζα του απορροφούμενου μαγγανίου ανά λίτρο απορροφούμενου νερού, ενώ ο εμβολιασμός της ‘Sammy’ στα υποκείμενα αυτά δεν είχε καμία επίδραση στην συγκέντρωση απορρόφησης μαγγανίου. Η συγκέντρωση απορρόφησης βορίου επηρεάστηκε από το συνθήκες θερμοκρασίας των θαλάμων και από τους συνδυασμούς των υποκειμένων/εμβολίων. Η πιπεριά είναι φυτό θερμής περιόδου με υψηλή ευαισθησία σε υποβέλτιστες θερμοκρασίες και συνεπώς η μείωση της παραγωγής καρπών που μετρήθηκε και οφείλονταν στην επίδραση των χαμηλών θερμοκρασιών στο συγκεκριμένο πείραμα αναμενόταν πλήρως. Επίσης, δεν παρατηρήθηκε αλληλεπίδραση μεταξύ της κατασταλτικής επίδρασης των χαμηλών θερμοκρασιών στην παραγωγής καρπών με τον εμβολιασμό και το υποκείμενο, πράγμα που δείχνει ότι τα υπό έλεγχο υποκείμενα δεν επηρεάζουν την ανεκτικότητα της πιπεριάς στο ψύχος.

Τα αποτελέσματα του συγκεκριμένου πειράματος έδειξαν ότι τόσο το υποκείμενο ‘Robusto’ όσο και το ‘Terrano’ αύξησαν τη συνολική παραγωγή καρπών κατά 39% και 34%, αντίστοιχα, σε σύγκριση με τους αυτό-εμβολιαζόμενους συνδυασμούς, όταν το εμβόλιο ήταν η εμπορική ποικιλία ‘Sammy’. Εντούτοις, όταν το εμβόλιο ήταν η εμπορική ποικιλία ‘Orangery’, μόνο το ‘Terrano’ αύξησε τη συνολική παραγωγή καρπών κατά 35% σε σύγκριση με εκείνη των αυτό-εμβολιαζόμενων φυτών ‘Orangery’, ενώ το ‘Robusto’ δεν είχε καμία επίδραση στην συνολική παραγωγή καρπών.Τα παραπάνω σημαντικά ευρήματα δείχνουν ότι η έρευνα για τον αγρονομικό αντίκτυπο του εμβολιασμού στις καλλιέργειες πιπεριάς θα πρέπει να βασίζεται στη δοκιμή κάθε μεμονωμένου συνδυασμού υποκειμένου/εμβολίου, παρά στη επιλογή διαφορετικών υποκειμένων χρησιμοποιώντας μόνο ένα εμβόλιο. Στη ‘Sammy’, η απουσία οποιασδήποτε επίδρασης του εμβολιασμού στη συνολική βιομάζα των φυτών και στην κατανάλωση νερού, παρά τη σημαντική αύξηση του αριθμού καρπών ανά φυτό, δείχνει την θετική επίδραση και των δύο υπό έλεγχο υποκειμένων στη καρπόδεση παρά στη ζωηρότητα των φυτών. Ωστόσο, όταν η ‘Orangery’ στο συγκεκριμένο πείραμα εμβολιάστηκε στο ‘Terrano’, τόσο η φυτική βιομάζα όσο και η κατανάλωση νερού αυξήθηκαν αναλογικά με την αύξηση του αριθμού καρπών ανά φυτό, κάτι που δείχνει ότι αυτός ο συνδυασμός εμβολιασμού αυξάνει την παραγωγή λόγω της αύξησης της ζωηρότητας των φυτών. Ωστόσο, στο συγκεκριμένο πείραμα, όλοι οι συνδυασμοί εμβολιασμού αύξησαν την παραγωγή αποκλειστικά μέσω της αύξηση του αριθμού καρπών ανά φυτό, ενώ δεν είχαν καμία επίδραση στο μέσο βάρος των καρπών. Εξ ορισμού, η επίδραση του εμβολιασμού στην αποδοτικότητα χρήσης νερού εξαρτάται τόσο από την παραγωγή καρπών όσο και από την κατανάλωση νερού. Έτσι, μερικές φορές οι συνδυασμοί υποκειμένων/εμβολίων επιβάλλουν πολύ πιο διαφορετικές επιδράσεις στην παραγωγή καρπών, παρά στην κατανάλωση νερού, που μπορεί οι επιδράσεις αυτές τελικώς να έχουν παρόμοιες επιπτώσεις στην αποδοτικότητα χρήσης νερού. Πράγματι, ο εμβολιασμός της 'Orangery' στο 'Robusto' δεν είχε αντίκτυπο στην αποδοτικότητα χρήσης νερού επειδή δεν είχε αντίκτυπο τόσο στην παραγωγή καρπών όσο και στην κατανάλωση νερού. Εντούτοις, ο εμβολιασμός της 'Orangery' στο 'Terrano' δεν είχε επίδραση στην αποδοτικότητα χρήσης νερού, επειδή αυτός ο συνδυασμός υποκειμένου/εμβολίου αύξησε αναλογικά τόσο την παραγωγή καρπών όσο και την κατανάλωση νερού, καθώς ενίσχυσε την παραγωγή βιομάζας στο βλαστικό στάδιο. Επιπλέον, ο εμβολιασμός της 'Sammy' αύξησε την αποδοτικότητα χρήσης νερού ανεξάρτητα από το υπό δοκιμή υποκείμενα, επειδή τόσο το 'Robusto' όσο και το 'Terrano' αύξησαν την παραγωγή καρπών χωρίς να επηρεάσουν την παραγωγή βιομάζας στο βλαστικό στάδιο και ταυτόχρονα την κατανάλωση νερού μέσω της διαπνοής. Αυτά τα αποτελέσματα υποδεικνύουν ότι η επίδραση του εμβολιασμού στην αποδοτικότητα χρήσης νερού εξαρτάται από τον συνδυασμό υποκειμένου/εμβολίου και όχι μόνο από το υποκείμενο ή το εμβόλιο.

Στο συγκεκριμένο πείραμα το μοναδικό αβιοτικό στρές που δοκιμάστηκε, δηλαδή η χαμηλή θερμοκρασία, δεν αλληλεπίδρασε με κανέναν συνδυασμό εμβολιασμού. Ωστόσο, οι μετρήσεις της παραγωγής φυτικής βιομάζας, της κατανάλωσης νερού και των συγκεντρώσεων απορρόφησης θρεπτικών στοιχείων, επιτρέπουν μια εκτίμηση της συμβολής της απορρόφησης θρεπτικών στοιχείων και νερού στην αύξηση της παραγωγής καρπών. Έτσι, η θετική επίδραση του εμβολιασμού στην παραγωγή καρπών στις τυπικές θερμοκρασίες του θαλάμου 1 του συγκεκριμένου πειράματος, θα μπορούσε εν μέρει να συνδεθεί με αυξημένες συγκεντρώσεις απορρόφησης καλίου και μαγνησίου (για το 'Terrano') ή μαγνησίου (για το 'Robusto'). Η αύξηση όμως της παραγωγής καρπών σε ορισμένους συνδυασμούς εμβολιασμού παρατηρήθηκε και στα δύο καθεστώτα θερμοκρασίας που εξετάστηκαν, ενώ οι αυξήσεις των συγκεντρώσεων απορρόφησης καλίου και μαγνησίου παρατηρήθηκαν μόνο στα φυτά που καλλιεργήθηκαν υπό τυπικές συνθήκες θερμοκρασίας. Επιπλέον, οι συγκεντρώσεις απορρόφησης των ασβεστίου, αζώτου, φωσφόρου και των μικροστοιχείων δεν αυξήθηκαν από οποιοδήποτε συνδυασμό εμβολιασμού. Έτσι, μια ευνοϊκή ρύθμιση της αποτελεσματικότητας απορρόφησης των θρεπτικών στοιχείων δεν μπορεί να θεωρηθεί ο σημαντικότερος λόγος για την αύξηση της παραγωγής καρπών που προκύπτει από ορισμένους συνδυασμούς εμβολιασμού. Απαιτούνται περαιτέρω έρευνες για να αποκαλυφθούν οι φυσιολογικοί και μοριακοί μηχανισμοί που εμπλέκονται στην ενίσχυση της συνολικής παραγωγής καρπών ως αποτέλεσμα ορισμένων συνδυασμών υποκειμένου/εμβολίου.

Στην παρούσα μελέτη, τόσο το ‘Robusto’ όσο και το ‘Terrano’ όταν εμβολιάστηκαν στην ‘Orangery’μείωσαν ελαφρώς αλλά σημαντικά την απορρόφηση μαγγανίου, ενώ τα ίδια αυτά υποκείμενα δεν επηρέασαν την συγκέντρωση απορρόφησης μαγγανίου όταν το εμβόλιο ήταν η ‘Sammy’. Στο προηγούμενο πείραμα επίσης δεν βρέθηκε καμία επίδραση του εμβολιασμού στην συγκέντρωση απορρόφησης μαγγανίου. Οι διαφορετικές επιδράσεις των ‘Sammy’ και ‘Orangery’ στον εμβολιασμό, σχετικά με την συγκέντρωση απορρόφησης μαγγανίου, υποστηρίζουν περαιτέρω την αντίληψη ότι η επίδραση του εμβολιασμού στις συγκεντρώσεις απορρόφησης των θρεπτικών στοιχείων εξαρτάται από κάθε συγκεκριμένο συνδυασμό υποκειμένου/εμβολίου και όχι από τον γονότυπο του υποκειμένου. Οι αυξήσεις στις συγκεντρώσεις απορρόφησης καλίου, ασβεστίου, μαγνησίου και αζώτου στην μεταχείριση χαμηλών θερμοκρασιών, ήταν αποτέλεσμα της μείωσης της κατανάλωσης νερού κατά 24%. Ωστόσο η μείωση της συγκέντρωση απορρόφησης φωσφόρου στις χαμηλές θερμοκρασίες υποδεικνύει ότι ο μηχανισμός απορρόφησης των H_2PO_4 στην πιπεριά είναι πολύ πιο ευαίσθητος στις χαμηλές θερμοκρασίες από εκείνου του καλίου, ασβεστίου, μαγνησίου και αζώτου. Η συγκέντρωση απορρόφησης φωσφόρου ανταποκρίνεται πολύ ισχυρότερα στη θερμοκρασία της ρίζας από τις αντίστοιχες συγκεντρώσεις απορρόφησης των αζώτου και καλίου. Οι συνθήκες χαμηλών θερμοκρασιών είχαν ως αποτέλεσμα να υπάρχουν χαμηλές θερμοκρασίες στη ζώνη της ρίζας, οι οποίες θερμοκρασίες ανακτήθηκαν βραδύτερα κατά τη διάρκεια της ημέρας από ότι η αντίστοιχη θερμοκρασία του αέρα. Η μεγάλη μείωση της συγκέντρωση απορρόφησης φωσφόρου στις χαμηλές θερμοκρασίες στην πιπεριά, μπορεί να αποδοθεί κυρίως στη χαμηλή θερμοκρασία της ρίζας. Από μια άλλη πλευρά, τα αποτελέσματα του συγκεκριμένου πειράματος υποστηρίζουν την άποψη ότι η συγκέντρωση απορρόφησης φωσφόρου στην πιπεριά επηρεάζεται ισχυρότερα από τη θερμοκρασία της ρίζας, παρά από τη θερμοκρασία που επικρατεί στον αέρα. Οι μέσες τιμές συγκεντρώσεων απορρόφησης που βρέθηκαν στο συγκεκριμένο πείραμα κατά τη διάρκεια της βλαστικής περιόδου καλλιέργειας υπό συνθήκες τυπικών θερμοκρασιών ήταν 2.92 mmol L^{-1} ασβέστιο, 0.92 mmol L^{-1} μαγνήσιο, 6.03 mmol L^{-1} κάλιο, 12.14 mmol L^{-1} άζωτο και 0.89 mmol L^{-1} φώσφορο.

Στην τρίτη πειραματική εργασία, μελετήθηκε σε πείραμα καλλιέργειας πιπεριάς σε υαλόφρακτο θερμοκήπιο η επίδραση της αλατότητας που προκαλείται από το ασβέστιο, μιας και αντίστοιχες επιστημονικές μελέτες δεν υπάρχουν και αυτό παρά το γεγονός ότι σε πολλές περιοχές παγκοσμίως υπάρχει παρουσία υψηλότερων συγκεντρώσεων $Ca(HCO_3)_2$ από αυτές του χλωριούχου νατρίου σε εδάφη, υπόγεια ύδατα και επιφανειακά ύδατα. Για να εκτιμηθεί επίσης η υπόθεση ότι οι συγκεντρώσεις απορρόφησης μπορεί να επηρεαστούν όταν μια καλλιέργεια αναπτύσσεται κάτω από διαφορετική ποιότητα νερού άρδευσης, προσδιορίστηκαν οι συγκεντρώσεις απορρόφησης των αζώτου, θείου, καλίου, ασβεστίου και μαγνησίου υπό συνθήκες προοδευτικής συσσώρευσης Ca στα ανακυκλούμενο θρεπτικό διάλυμα και συνεπώς στη ζώνη της ρίζας των φυτών. Επιπλέον, μελετήθηκε η επίδραση των υπερβολικά υψηλών συγκεντρώσεων Ca στη ζώνη της ρίζας τόσο στην παραγωγή βιομάζας όσο και στην παραγωγή και ποιότητα των καρπών.

Το πείραμα διεξήχθη σε ένα θερμαινόμενο θάλαμο θερμοκηπίου στον οποίο εφαρμόστηκαν τέσσερις πειραματικές μεταχειρίσεις που αντιστοιχούσαν σε τέσσερις διαφορετικές συγκεντρώσεις ασβεστίου στο νερό άρδευσης και συγκεκριμένα 1.5, 3.0, 4.5 και 6.0 mM. Κάθε μεταχείριση κατανεμήθηκε τυχαία στο θερμοκήπιο σε πέντε κλειστά υδροπονικά συστήματα και έτσι χρησιμοποιήθηκαν συνολικά 20 πειραματικά κλειστά υδροπονικά συστήματα. Τα φυτά της πιπεριάς (*Capsicum annuum* L.) της ποικιλίας ‘Sammy’ η οποία παράγει επιμήκη τύπου καρπούς, αναπτύχθηκαν σε θάλαμο θερμοκηπίου εντός ανακυκλούμενου θρεπτικού διαλύματος, σύμφωνα με τις αρχές της καλλιέργειας σε λεπτή στοιβάδα θρεπτικού διαλύματος (Nutrient Film Technique-NFT). Εννέα σπορόφυτα μεταφέρθηκαν σε κάθε κλειστό υδροπονικό σύστημα στο στάδιο των 5-6 αληθινών φύλλων. Η ανακύκλωση της απορροής ξεκίνησε στις 11 Σεπτεμβρίου και διήρκεσε μέχρι τις 25 Φεβρουαρίου.

Οι συγκεντρώσεις θρεπτικών στοιχείων στο αρχικό θρεπτικό διάλυμα ήταν σε όλες τις μεταχείρισεις οι ακόλουθες: 5,5 mM K, 6,5 mM Ca, 2,0 mM Mg, 1,1 mM NH_4, 16,4 mM NO_3, 1,2 mM H_2PO_4, 5,8 mM SO4, 20,0 μM Fe, 10,0 μM Mn, 6.0 μM Zn, 0.8 μM Cu, 50.0 μM B και 0.5 μM Mo. Μετά την μεταφύτευση, τα θρεπτικά στοιχεία και το νερό που απορροφήθηκαν από τα φυτά συμπληρώνονταν καθημερινά με την προσθήκη νέου θρεπτικού διαλύματος συμπλήρωσης, με συγκεντρώσεις (εκτός από εκείνες του ασβεστίου) που υποτίθεται ότι αντιστοιχούν στις αναλογίες απρρόφησης θρεπτικών στοιχείων σε νερό σύμφωνα με τα ευρήματα της έρευνας της πρώτης πειραματικής εργασίας. Οι συγκεντρώσεις ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης ρυθμίστηκαν σε τέσσερα διαφορετικά επίπεδα (1.5, 3.0, 4.5 και 6.0 mM) που αντιστοιχούσαν στις τέσσερις μεταχειρίσεις. Αμέσως μετά τη μεταφύτευση που πραγματοποιήθηκε στις 11 Σεπτεμβρίου, συλλέχθηκαν δείγματα αρχικών θρεπτικών διαλυμάτων από όλες τις κλειστές μονάδες υδροπονικών συστημάτων.

Η συσωρευτική ποσότητα κατανάλωσης νερού των φυτών πιπεριάς στο τέλος της καλλιέργειας μετρήθηκε 15%, 19% και 28% λιγότερη στα φυτά που αναπτύχθηκαν στις μεταχειρίσεις με 3.0, 4.5 και 6.0 mM ασβέστιο στο θρεπτικό διάλυμα αντίστοιχα, σε σύγκριση με τη μεταχείριση ελέγχου που ήταν 1.5 mM ασβέστιο. Η αλατότητα στο ανακυκλωμένο θρεπτικό διάλυμα σταδιακά αυξήθηκε με το χρόνο ανάλογα με τις συγκεντρώσεις ασβεστίου στα θρεπτικά διαλύματα, ενώ αντίστροφη ήταν η περίπτωση της μεταχείρισης ελέγχου στην οποία η αλατότητα στο νερό απορροής μειώθηκε ελαφρώς.

Οι διαφορετικές μεταχειρίσεις τροφοδοσίας ασβεστίου δεν είχαν σημαντική επίδραση στο μέσο βάρος των καρπών ανά φυτό, αν και επηρεάστηκε έντονα η συνολική παραγωγή καρπών και ο αριθμός καρπών ανά φυτό. Ειδικότερα, ο συνολικός αριθμός καρπών στη μεταχείριση με τη χαμηλότερη συγκέντρωση ασβεστίου (δηλαδή 1.5 mM) στο θρεπτικό διάλυμα συμπλήρωσης ήταν 22% υψηλότερος από την μεταχείριση 3.0 mM, 26% υψηλότερος από την μεταχείριση 4.5 mM και 30% υψηλότερος από την μεταχείριση 6.0 mM. Συνεπώς, το συνολικό βάρος καρπών στη μεταχείριση με τη χαμηλότερη συγκέντρωση ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης (δηλ. 1.5 mM) ήταν 24% υψηλότερο από ό, τι στην μεταχείριση 3.0 mM, 34% υψηλότερη από ότι στην 4.5 mM και 32% υψηλότερη από ό, τι στην μεταχείριση 6.0 mM. Ωστόσο, οι διαφορετικές μεταχειρίσεις παροχής ασβεστίου δεν είχαν σημαντική επίδραση στην αποτελεσματικότητα χρήσης νερού.

Επιπλέον, οι συγκεντρώσεις ασβεστίου στις μεταχειρίσεις με 3.0, 4.5 ή 6.0 mM ασβέστιο στο θρεπτικό διάλυμα αυξήθηκαν κατά τη διάρκεια ολόκληρης της πειραματικής περιόδου έως το στάδιο της συγκομιδής, όπου τα ποσοστά συσσώρευσης έδειξαν τάση σταθεροποίησης σε ένα μέγιστο επίπεδο και αναλογικά με τη συγκέντρωση ασβεστίου στο θρεπτικό διάλυμα. Αντίθετα με τα παραπάνω, η συγκέντρωση ασβεστίου στο θρεπτικό διάλυμα που παρείχε 1.5 mM ασβέστιο μειώθηκε ελαφρώς κατά την έναρξη της ανθήσεως, ενώ κατά την περίοδο της συγκομιδής τα ποσοστά συσσώρευσης ασβεστίου έδειξαν τάση σταθεροποίησης σε ένα ελάχιστο επίπεδο. Τα ιόντα ασβεστίου που συσσωρεύτηκαν στο διάλυμα απορροής ανήλθαν μέχρι 2.5, 17.2, 27.7 και 37.2 mM, στις μεταχειρίσεις με συγκεντρώσεις ασβεστίου 1.5, 3.0, 4.5 και 6.0 mM στο θρεπτικό διάλυμα, αντίστοιχα. Στα φύλλα, οι συγκεντρώσεις ασβεστίου αυξήθηκαν σε όλες τις μεταχειρίσεις με την αύξηση της συγκέντρωσης ασβεστίου στο παρεχόμενο θρεπτικό διάλυμα. Οι συγκέντρωσεις απορρόφησης ασβεστίου αυξήθηκαν με το χρόνο όταν οι συγκεντρώσεις ασβεστίου στο θρεπτικό διάλυμα ήταν 4.5 ή 6.0 mM, ενώ η συγκέντρωση απορρόφησης ασβεστίου μειώθηκε ελαφρά όταν οι συγκεντρώσεις ασβεστίου στο θρεπτικό διάλυμα ήταν 1.5 ή 3.0 mM.

Τα νιτρικά, και επομένως η ολική συγκέντρωση αζώτου στο θρεπτικό διάλυμα, αυξήθηκαν κατά την πειραματική περίοδο μόνο όταν οι συγκεντρώσεις ασβεστίου ήταν 3.0, 4.5 και 6.0 mM στο θρεπτικό διάλυμα συμπλήρωσης. Ωστόσο, ο ρυθμός απορρόφησης ολικού αζώτου μειώθηκε ελαφρά μετά την έναρξη της πειραματικής περιόδου και όταν η συγκέντρωση ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης ήταν 1.5 mM, με τάση να σταθεροποιείται προς το τέλος του πειράματος σε ένα ελάχιστο επίπεδο. Οι συγκεντρώσεις του ολικού αζώτου στα φύλλα μειώθηκαν με το χρόνο, ανεξάρτητα από τις μεταχειρίσεις παροχής ασβεστίου. Οι μέσες συγκεντρώσεις απορρόφησης του ολικού αζώτου κατά τη διάρκεια ολόκληρης της πειραματικής περιόδου κυμαίνονταν μεταξύ 11.0 – 11.7 mmol L^{-1}, 12.2-13.4 mmol L^{-1}, 13.2-14.2 mmol L^{-1} και 14.2 – 15.7 mmol L^{-1} στις μεταχειρίσεις με συγκεντρώσεις ασβεστίου 1.5, 3.0, 4.5 και 6.0 mM στο θρεπτικό διάλυμα, αντίστοιχα.

Οι συγκεντρώσεις των θεϊκών αυξήθηκαν προοδευτικά κατά τη διάρκεια της πειραματικής περιόδου στο θρεπτικό διάλυμα που τροφοδοτήθηκε με 3.0, 4.5 ή 6.0 mM ασβέστιο μέχρι το τέλος του πειράματος, ενώ αντίθετα οι συγκεντρώσεις των θεϊκών στο θρεπτικό διάλυμα τροφοδοσίας με 1.5 mM ασβέστιο ήταν σταθερές καθ' όλη τη διάρκεια της πειραματικής περιόδου. Ωστόσο, οι συγκεντρώσεις του θείου στα φύλλα μειώθηκαν στο στάδιο άνθησης ανεξάρτητα από τις εφαρμοζόμενες μεταχειρίσεις και στην συνέχεια έως το τέλος της περιόδου συγκομιδής αυξήθηκαν, ακολουθώντας τις διαφορετικές συγκεντρώσεις των θεϊκών στα θρεπτικά διαλύματα των μεταχειρίσεων. Οι μέσες συγκεντρώσεις απορρόφησης των θεϊκών αυξήθηκαν, ακολουθώντας τις διαφορετικές συγκεντρώσεις των θεϊκών στα θρεπτικά διαλύματα των μεταχειρίσεων.

Η συγκέντρωση καλίου ήταν χαμηλότερη όταν η συγκέντρωση ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης ήταν 1.5 mM σε σύγκριση με όλες τις άλλες μεταχειρίσεις. Επιπλέον οι μέσες συγκεντρώσεις καλίου ανήλθαν σε 70.7 mg (g DW) $^{-1}$ στα φύλλα, ενώ η μέση συγκέντρωση απορρόφησης καλίου (7.0 mmol L^{-1}) δεν διέφερε σημαντικά μεταξύ των μεταχειρίσεων και παρέμεινε στα ίδια επίπεδα καθ' όλη τη διάρκεια της πειραματικής περιόδου. Οι μέσες συγκεντρώσεις απορρόφησης του καλίου δεν διέφεραν σημαντικά μεταξύ των μεταχειρίσεων και διατηρήθηκαν στα ίδια επίπεδα καθ΄ όλη την πειραματική περίοδο.

Η συγκέντρωση μαγνησίου ήταν σημαντικά χαμηλότερη στην χαμηλότερη συγκέντρωση ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης σε σύγκριση με τις άλλες τρεις μεταχειρίσεις τροφοδοσίας ασβεστίου (3.0, 4.5 ή 6.0 mM ασβέστιο). Τα επίπεδα μαγνησίου δεν μεταβλήθηκαν καθ΄ όλη την πειραματική περίοδο όταν τα φυτά αναπτύχθηκαν στην μεταχείριση με 1.5 mM ασβέστιο στο ανακυκλούμενο θρεπτικό διάλυμα. Αντίθετα, η συγκέντρωση μαγνησίου στα φύλλα όλων των άλλων μεταχειρίσεων μειώθηκε με το χρόνο, χωρίς να υπάρχουν στατιστικά σημαντικές διαφορές μεταξύ των. Οι μέσες τιμές συγκεντρώσεων απορρόφησης μαγνησίου δεν επηρεάστηκαν από τις μεταχειρίσεις μέχρι την έναρξη της συγκομιδής, ωστόσο ήταν σημαντικά υψηλότερες μετά από το στάδιο αυτό στη μεταχείριση ελέγχου με 1,5 mM ασβέστιο στο θρεπτικό διάλυμα συμπλήρωσης, σε σύγκριση με τις υπόλοιπες μεταχειρίσεις.

Η παρούσα μελέτη έδειξε ότι επίπεδα ασβεστίου έως 15 mmol L^{-1} στη ζώνη της ρίζας ως αποτέλεσμα συσσώρευσης από την εφαρμογή μεταχείρισης 3.0 mM ασβεστίου στο ανακυκλούμενο θρεπτικό διάλυμα, μείωσαν την παραγωγή καρπών κατά 32% περίπου, με την μείωση αυτή ωστόσο να μην είναι ανάλογη της αύξησης της αλατότητας. Και αυτό καθώς μια περαιτέρω αύξηση των επιπέδων ασβεστίου στην ρίζα στα περίπου 30 ή 38 mmol L^{-1} όταν οι συγκεντρώσεις ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης ήταν 4.5 ή 6.0 mM, αντίστοιχα, δεν μείωσε περαιτέρω την παραγωγή καρπών. Ωστόσο, το ασβέστιο από μόνο του δεν είναι τοξικό για τα φυτά, με την ζημία που προκαλείται από τις υπερβολικές εξωτερικές συγκεντρώσεις ασβεστίου να αποδίδεται κυρίως στις οσμωτικές επιδράσεις της αλατότητας και στην βλαπτική ανταγωνιστικότητα των καλίου, μαγνησίου και / ή των συγκεντρώσεων απορρόφησης μεταλλικών μικροστοιχείων, που έχει ως αποτέλεσμα ανισορροπίες θρεπτικών στοιχείων. Έτσι, η έλλειψη αναλογικότητας μεταξύ της μείωσης της παραγωγής καρπών και της αύξησης των επιπέδων αλατότητας (και ασβεστίου) στη ζώνη της ρίζας των φυτών πιπεριάς, φαίνεται να συνδέεται με ανισορροπίες θρεπτικών στοιχείων που προφανώς συνέβησαν ακόμη και όταν το επίπεδο ασβεστίου στο θρεπτικό διάλυμα που περιβάλλει τις ρίζες ήταν 15 και 30 mM.

Στην παρούσα μελέτη, μέτριες και υψηλές συγκεντρώσεις ασβεστίου (3.0, 4.5 και 6.0 mM) στο παρεχόμενο θρεπτικό διάλυμα αύξησαν τις συγκεντρώσεις ασβεστίου στο ανακυκλούμενο θρεπτικό διάλυμα στα 17.2, 27.7 και 37.2 mmol L^{-1} αντίστοιχα. Ως αποτέλεσμα, η ολική αλατότητα αυξήθηκε στη ζώνη της ρίζας σε υψηλότερα επίπεδα (6.4, 9.0 και 10.8 dS m^{-1}, αντίστοιχα) από το συνιστώμενο όριο 2.8 dS m^{-1} όταν η πιπεριά καλλιεργείται υδροπονικά και εκτίθεται σε αλατότητα χλωριούχου νατρίου. Αυτό το αποτέλεσμα υποδεικνύει ότι οι συγκεντρώσεις απορρόφησης ασβεστίου σε αυτές τις μεταχειρίσεις θα ήταν χαμηλότερες από τις συγκεντρώσεις ασβεστίου στα αντίστοιχα ανακυκλούμενα θρεπτικά διαλύματα, κάτι που στο συγκεκριμένο πείραμα παρατηρήθηκε κατά την μεγαλύτερη διάρκεια της καλλιεργητικής περιόδου.

Η μειωμένη κατανάλωση νερού που παρατηρήθηκε στα φυτά των μεταχειρίσεων με 3.0, 4.5 και 6.0 mM ασβέστιο στο θρεπτικό διάλυμα συμπλήρωσης, σε σύγκριση με εκείνη που καταγράφηκε στα φυτά που υποβλήθηκαν στη μεταχείριση ελέγχου με 1.5 mM ασβέστιο, οφείλεται στο γεγονός ότι τα φυτά που υφίστανται καταπόνηση εξαιτίας υπερβολικού ασβεστίου αναπτύσσουν λιγότερη φυλλική επιφάνεια από εκείνα της μεταχείρισης ελέγχου με 1.5 mM ασβέστιο και έτσι μειώνεται η συνολική τους φυτική διαπνοή. Η μειωμένη φυλλική επιφάνεια, που υποδηλώνει περιορισμό της βλαστικής ανάπτυξης σε φυτά που τροφοδοτούνταν με υψηλότερες συγκεντρώσεις ασβεστίου από 15 mM στη ζώνη της ρίζας (δηλ. 3.0 ή μεγαλύτερη από 3.0 mM ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης), ήταν πιθανώς ο κυριότερος παράγοντας για τον μικρότερο αριθμό καρπών ανά φυτό σε αυτές τις μεταχειρίσεις. Επιπλέον, ο περιορισμός σε παρόμοια επίπεδα της βλαστικής ανάπτυξης και της παραγωγής καρπών στις τρεις υψηλότερου ασβεστίου μεταχειρίσεις στο θρεπτικό διάλυμα μπορεί να συσχετιστεί με τη μείωση της συγκέντρωσης μαγνησίου των φύλλων σε αυτές τις μεταχειρίσεις. Και αυτό διότι η μείωση του μαγνησίου στα φύλλα ήταν επίσης παρόμοια στις παραπάνω αυτές μεταχειρίσεις συγκρινόμενη με την μέτρηση μαγνησίου στα φύλλα στη μεταχείριση χαμηλού ασβεστίου, με 1.5 mM ασβέστιο στο θρεπτικό διάλυμα συμπλήρωσης. Η παρόμοια αποτελεσματικότητα χρήσης νερού σε όλες τις μεταχειρίσεις προέρχεται πιθανώς από την παρόμοια μείωση της παραγωγής καρπών και της κατανάλωσης νερού από την αλατότητα του ασβεστίου.

Οι συγκέντρωσεις απορρόφησης ασβεστίου επηρεάστηκαν από τη συγκέντρωση ασβεστίου στο ανακυκλούμενο θρεπτικό διάλυμα, η οποία εξαρτάται τόσο από τη συγκέντρωση ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης όσο και από το στάδιο ανάπτυξης του φυτού. Ωστόσο, η επίπτωση του σταδίου ανάπτυξης του φυτού στη συγκέντρωση απορρόφησης του ασβεστίου ήταν εμφανής μόνο στη μεταχείριση χαμηλού ασβεστίου (1.5 mM ασβέστιο στο θρεπτικό διάλυμα συμπλήρωσης). Στις τρεις μεταχειρίσεις με υψηλή περιεκτικότητα σε ασβέστιο, η επίδραση του σταδίου ανάπτυξης του φυτού αλληλεπιδρά με την επίδραση της συγκέντρωσης ασβεστίου στο ανακυκλούμενο θρεπτικό διάλυμα η οποία τείνει να αυξάνεται λόγω της συσσώρευσης, προκαλώντας με τον τρόπο αυτό αντίστοιχες αυξήσεις στη συγκέντρωση απορρόφησης ασβεστίου. Στη μεταχείριση με χαμηλή περιεκτικότητα σε ασβέστιο δεν σημειώθηκε συσσώρευση ασβεστίου με την πάροδο του χρόνου και συνεπώς οι μεταβολές στις συγκεντρώσεις απορρόφησης ασβεστίου με την πάροδο του χρόνου οφείλονταν μόνο από την επίδραση του σταδίου ανάπτυξης του φυτού. Το μεγαλύτερο μέρος του ασβεστίου στα φυτά απαιτείται για τη σταθεροποίηση των κυτταρικών τοιχωμάτων και της πλασματικής μεμβράνης. Ως εκ τούτου, η επικράτηση των νέων αναπτυσσόμενων φύλλων κατά τη διάρκεια του πρώιμου βλαστικού σταδίου τα οποία εμφανίζουν έντονη κυτταρική διαίρεση, καταλήγει λογικά σε υψηλότερες απαιτήσεις ασβεστίου σε σχέση με τα τελευταία στάδια ανάπτυξης όπου υπάρχουν πολλά ώριμα φύλλα τα οποία χαρακτηρίζονται κυρίως από κυτταρική διεύρυνση και όχι κυτταρική διαίρεση. Σε αντίθεση με τη συγκέντρωση απορρόφησης ασβεστίου, η συγκέντρωση απορρόφησης αζώτου ήταν πιο σταθερή με την πάροδο του χρόνου, αφού το άζωτο σε νιτρική μορφή εισέρχεται στα κύτταρα και συσσωρεύεται στα κενοτόπια, συμβάλλοντας στην ομοιόσταση των κυττάρων ενεργώντας ως ένα ενεργειακά φθηνό οσμωτικό. Έτσι, οι αναλογίες απορρόφησης αζώτου σε νιτρική μορφή δεν μειώνονται με το χρόνο καθώς τα φυτά πιπεριάς αναπτύσσουν πολλά ώριμα φύλλα.

Η μετρούμενη συγκέντρωση του ασβεστίου στη ζώνη ρίζας ήταν περίπου 4 mM, όταν η συγκέντρωση απορρόφησης ασβεστίου κυμάνθηκε από 1,5 έως 2,3 mmol L^{-1}. Είναι γνωστό ότι οι συγκεντρώσεις ιόντων στο περιβάλλον της ρίζας διαφέρουν κατά πολύ από τις αντίστοιχες συγκεντρώσεις απορρόφησης και ειδικά για τα δισθενή ιόντα όπως το ασβέστιο, το μαγνήσιο και τα θεϊκά οι συγκεντρώσεις στη ζώνη της ρίζας θα πρέπει να είναι πολύ υψηλότερες από τις αντίστοιχες συγκεντρώσεις απορρόφησης που θεωρούνται βέλτιστες για την καλλιέργεια φυτών. Στο πεπόνι, μια συγκέντρωση απορρόφησης ασβεστίου 1.5 mmol L^{-1} απαιτούσε συγκέντρωση ασβεστίου περίπου 4 mM στο περιβάλλον της ρίζας, η οποία είναι παρόμοια με τις τιμές που βρέθηκαν στην τρέχουσα μελέτη για την πιπεριά. Οι μεγαλύτερες εξωτερικές συγκεντρώσεις ασβεστίου στην καλλιέργεια πεπονιού επέβαλαν σημαντικά υψηλότερες συγκεντρώσεις ασβεστίου στη ριζική ζώνη, κάτι που βρίσκεται σε συμφωνία με τα αποτελέσματα της παρούσας μελέτης. Στη μεταχείριση χαμηλού ασβεστίου, η συγκέντρωση ασβεστίου στο ανακυκλωμένο θρεπτικό διάλυμα τείνει να μειώνεται με το χρόνο και αυτό επέβαλε μια αντίστοιχη μείωση στη συγκέντρωση απορρόφησης ασβεστίου. Αυτό το εύρημα δείχνει ότι μια συγκέντρωση 1.5 mM ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης είναι πιθανώς ανεπαρκής για την πιπεριά που αναπτύσσεται σε κλειστά υδροπονικά συστήματα. Παρόλα αυτά, καθώς η συγκέντρωση 3.0 mM στο ανακυκλούμενο θρεπτικό διάλυμα είχε ως αποτέλεσμα τη συσσώρευση ασβεστίου, η βέλτιστη συγκέντρωση ασβεστίου στο ανακυκλούμενο θρεπτικό διάλυμα για την πιπεριά που καλλιεργείται σε κλειστά υδροπονικά συστήματα υπό κλιματολογικές συνθήκες της Μεσογείου θα πρέπει να είναι μεταξύ 1.5 και 3.0 mM και πιο κοντά στο 1.5 αντί στο 3.0 mM. Στην τρέχουσα μελέτη, η αύξηση της συγκέντρωσης ασβεστίου στο θρεπτικό διάλυμα συμπλήρωσης που χορηγήθηκε στις διαφορετικές μεταχειρίσεις εξισορροπήθηκε ηλεκτροχημικά με αντίστοιχες αυξήσεις στις συγκεντρώσεις νιτρικών και θεϊκών.

Εν τούτοις, η αύξηση των συγκεντρώσεων νιτρικών και θεϊκών από 11.5 και 0.7 mM, αντίστοιχα, στο ανακυκλούμενο θρεπτικό διάλυμα, έως 13.5 και 1.2 mM ή υψηλότερη αντίστοιχα, είχε σαν αποτέλεσμα την βαθμιαία συσσώρευση των συγκεντρώσεών τους στο ανακυκλούμενο θρεπτικό διάλυμα, κάτι που δεν είναι επιθυμητό. Έτσι, οι συγκεντρώσεις νιτρικών και θεϊκών στο ανακυκλούμενο θρεπτικό διάλυμα που παρέχονται στην πιπεριά που αναπτύσσεται σε κλειστά

υδροπονικά συστήματα πρέπει να είναι χαμηλότερες από 13.5 και 1.2 mM, αντίστοιχα. Παρ 'όλα αυτά, οι συγκεντρώσεις νιτρικών και θεϊκών στο ανακυκλούμενο θρεπτικό διάλυμα που παρέχονται στη μεταχείριση με χαμηλή περιεκτικότητα σε ασβεστίου (11.5 και 0.7 mM), σταδιακά κατέληξαν σε μείωση των επιπέδων νιτρικών και θεϊκών στο ανακυκλωμένο θρεπτικό διάλυμα, το οποίο επίσης δεν είναι επιθυμητό. Έτσι, για τις καλλιέργειες πιπεριάς που καλλιεργούνται σε κλειστές υδροπονικές καλλιέργειες τα βέλτιστα επίπεδα στο ανακυκλούμενο θρεπτικό διάλυμα θα πρέπει να κυμαίνονται μεταξύ 11.5 και 13.5 για νιτρικά και 0.7 και 1.2 για θεϊκά.

Συμπερασματικά, οι γνώσεις που αποκτήθηκαν από την έρευνα που διεξήχθη κατά την εκπόνηση της συγκεκριμένης διδακτορικής διατριβής δείχνουν ότι οι συγκεντρώσεις απορρόφησης των ασβεστίου, μαγνησίου, καλίου και αζώτου είναι γενικά υψηλότερες στις χειμερινές καλλιέργειες εκτός εδάφους πιπεριάς που αναπτύσσονται στις κλιματολογικές συνθήκες της Μεσογείου, από εκείνες τις συγκεντρώσεις απορρόφησης που αναφέρθηκαν σε ολλανδικά θερμοκήπια, ενώ αυτή του φωσφόρου είναι παρόμοια και στα δύο περιβάλλοντα. Επιπρόσθετα, η θερμοκρασία μέσα στο θερμοκήπιο, η ποικιλία και το υποκείμενο κατά τη χρήση εμβολιασμένων φυτών, μπορεί να έχουν σημαντική επίδραση στις συγκεντρώσεις απορρόφησης των θρεπτικών στοιχείων. Δεδομένου ότι αυτοί οι παράγοντες μπορεί να διαφέρουν σε διαφορετικές καλλιέργειες, οι συγκεντρώσεις απορρόφηση που βρέθηκαν είναι μόνο ενδεικτικές και η χρήση τους ως βάση για την παρασκευή θρεπτικών διαλυμάτων για κλειστά υδροπονικά συστήματα συνεπάγεται συχνή αναπροσαρμογή των τελευταίων, μετά από μετρήσεις των συγκεντρώσεων των θρεπτικών στοιχείων στη ζώνη της ρίζας. Τέλος σε καλλιέργειες πιπεριάς που αναπτύσσονται σε εκτός εδάφους κλειστά υδροπονικά συστήματα, η συγκέντρωση ασβεστίου στο νερό άρδευσης πρέπει να είναι χαμηλότερη από 3.0 mM για να αποφευχθούν μειώσεις στην ανάπτυξη φυτών και παραγωγή καρπών λόγω αλατότητας.

Λέξεις κλειδιά: *Capsicum annum* L., αβιοτικό στρές, άρδευση, υποκείμενο, ποικιλία

SUMMARY

The nutrient to water uptake ratios, henceforth termed “uptake concentrations” (UC), remain relatively constant over time under similar climatic conditions for a particular plant species and developmental stage. Under greenhouses with low temperature (LT) conditions, the uptake of nutrients may be altered in a different manner than that of the water and thus their UC may be different than in greenhouses with standard temperature (ST) conditions. In the Mediterranean regions, sweet pepper is frequently cultivated in unheated greenhouses in which the temperature during the winter may drop to suboptimal or even lower levels. In these areas, the available irrigation water frequently contains sodium chloride but also calcium bicarbonate, which at excessively high concentrations in closed hydroponic crops can impose Ca accumulation in the recycled NS and concomitantly negatively affect fruit yield and quality of the produce.

Taking the above into consideration there were established three studies:

In the first study, pepper plants of the cultivars ‘Sammy’ and ‘Orangery’, self-grafted or grafted onto two commercial rootstocks ('Robusto' and 'Terrano'), were cultivated in a greenhouse under either ST or LT conditions. The aim of the study was to test the impact of grafting and greenhouse temperature on total yield, water use efficiency and nutrient uptake. The LT regime reduced yield by about 50% in ‘Sammy’ and 33% in ‘Orangery’, irrespective of the grafting combination. Grafting of ‘Sammy’ onto both 'Robusto' and 'Terrano' increased the total fruit yield by 39% and 34% compared with the self-grafted control, while grafting of ‘Orangery’ increased yield only when the rootstock was ‘Terrano’. The yield increase resulted exclusively from enhancement of the fruit number per plant. Both the water consumption and the water use efficiency were suppressed by the LT regime but the temperature effect interacted with the rootstock/scion combination. The LT increased the UC of K, Ca, Mg, N, and Mn, while it decreased strongly that of P and slightly the UC of Fe, and Zn. The UC of K and Mg were influenced by the rootstock/scion combination but this effect interacted with the temperature regime. In contrast, the Ca, N, and P concentrations were not influenced by the grafting combination. The results of the present study show that the impact of grafting on yield and nutrient uptake in pepper depend not merely on the rootstock genotype but on the rootstock/scion combination.

In the second study, mean UC of macro- and micronutrients were determined during five developmental stages in different pepper cultivars grown in a closed hydroponic system by measuring the water uptake and the nutrient removal from the RNS. The experiment was conducted in a Mediterranean environment and the tested cultivars were ‘Orangery’, ‘Bellisa’, ‘Sondela’, ‘Sammy’ self-grafted and ‘Sammy’ grafted onto the commercial rootstock `RS10'. ‘Sondela’ exhibited significantly higher NO_3, Mg, Ca and B UC in comparison with all other cultivars, while Bellisa exhibited higher K UC. The UC of all nutrients were similar in the grafted and the non-grafted ‘Sammy’ plants. The UC of macronutrients estimated in the second study (mmol L^{-1}) ranged from 2.4 to 3.7 for Ca, 1.0 to 1.5 for Mg, 6.2 to 9.0 for K, 11.7 to 13.7 for N, and 0.7 to 1.1 for P. The UC of N, K, Ca and Mg were appreciably higher than the corresponding values found under Dutch climatic conditions, while that of P was similar in both environments during the vegetative stage and higher thereafter. The UC of Fe, Zn and B tended to decrease with time, while that of Mn increased initially and subsequently decreased slightly during the reproductive developmental stage.

In the third study, irrigation water containing 1.5, 3.0, 4.5 and 6.0 mM was used to prepare NS in a closed hydroponic crop of sweet pepper cultivated in RNS. The aim of the study was to determine maximum Ca levels that do not harm the crop and to simulate the pattern of Ca accumulation when the Ca concentration in the irrigation water is excessive. At 1.5 mM Ca, no Ca accumulation was observed in the RNS, while at 3.0, 4.5 and 6.0 mM the Ca concentration in the RNS, and concomitantly in the root environment, increased to 17, 28 and 37 mM, corresponding to 6.4, 9.0 and 10.8 dS m^{-1}. The accumulation of Ca in the RNS affected both tissue nutrient concentrations and UC of Ca, S and Mg, but this was not the case for N and K. Growth, yield and plant water uptake were restricted at moderate and high external Ca levels. Our results showed that in soilless sweet pepper crops with zero discharge of fertigation effluents, the Ca concentration in the irrigation water should be lower than 3.0 mM to avoid yield restrictions due to salinity.

Key words: *Capsicum annum* L., abiotic stress, irrigation, rootstock, cultivar

CHAPTER 1. GENERAL INTRODUCTION

Pepper (*Capsicum annuum* L. ssp. *Annuum*) is among the most widely cultivated horticultural crops with an estimated global production reaching over 38 million tons every year for both, chili pepper and sweet pepper (Faostat 2019). It is believed to originate from Central and South America, while can be produced year around in warm climates (Monteiro *et al.* 2009). It is a widespread species not only because of its economic importance but also due to the nutritional value of its fruits, which are characterized by high levels of antioxidants, such as ascorbic acid, carotenoids, β-carotene (pro-vitamin A) and phenolic compounds (Howard et al. 2000, Palma et al. 2009, Martí et al. 2011). In order to meet growing demand agricultural production by 2050, farmers need to improve and increase agricultural productivity by 60 percent from 2007 levels on a sustainable basis, while conserving and enhancing natural resources, such as water (Alexandratos and Bruinsma 2012). The high productivity of greenhouses plays an important role to meet the growing food demand worldwide and particularly for vegetables (Marcelis and Heuvelink 2019).

Sweet pepper is one of the important five high valuable crops in greenhouses. As plant organism, grow as a result of the influence of their genetics and their environment consisting of physical, chemical and biological factors. One aspect of this environment is physical micro-environment or microclimate, which is the assembly of the climatic parameters forming around living plants (Boonen *et al*. 2000). In greenhouses, the microclimate can be manipulated in order to provide appropriate environmental conditions for crops by control actions, such as shading (by screens or whitening), ventilation (natural or forced) and cooling (by fog or fan and pan systems) during summer, while during winter the main system used are heating, ventilation and carbon dioxide enrichment (Katsoulas and Kittas 2008). As the microclimatic conditions offered to the crop is directly associated with the production potential of a crop grown inside a protective structure, a better understanding of the relationships between sweet pepper plants and greenhouse microclimatic parameters is extremely important to offer most favourable conditions for improved plant growth and development under protective structures.

Temperature is one of the most important environmental factors influencing plant growth, development and yield. The temperature threshold for growth of most of the chilling-sensitive fruit vegetables, such as pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), tomato (*S. lycopersicum* L.) and melon (*C. melo, cucrbita*), is about 8–12°C (Hansen & Hara 1994, Criddle et al. 1997). As reported by Schwarz *et al.* (2010), each aspect of growth, development and/or fruit formation has its own temperature optimum, which varies among and within species as well with plant age.

Bakker and van Uffelen (1988) conducted a glasshouse experiment to investigate the effect of temperature on growth of sweet pepper (*C. annuum*). Twelve day/night temperature regimes (16/15, 16/21, 20/12, 20/15, 20/18, 20/21, 24/12, 24/15, 24/18, 24/21, 28/15 and 28/21°C) were applied. Fresh weight and leaf number showed a significant positive correlation with 24-h mean temperature. No significant effect of the day/night temperature amplitude was found. The optimum 24-h mean temperature for vegetative growth was obtained between 21 and 23°C. The effect of 24-h mean temperature on vegetative growth was of greater importance compared to the effect of the day/night temperature amplitude. Niu *et al.* (2006) studied the effect of drought and temperature on growth and leaf gas exchange of sweet pepper (*C. annuum*) under greenhouse conditions. They found that when the temperature is higher than 30°C, the rate of photosynthesis decreased.

Li *et al*. (2015) described that the optimal temperature range for pepper growth is 20–30°C, since greater heat reduced considerably the vegetative and reproductive growth phases of pepper (Kafizadeh *et al*. 2008). Wien (1997) and Rubatzky and Yamaguchi (1999) were obtained similar results. Mercado *et al.* (1997a) reported that the optimum growth temperature is between 25 and 30°C, in such a way that temperature changes affect a variety of physiological functions and morphological development. Pepper growth is reduced when temperature decreases below 15 °C.

The most important factor determining flower differentiation is air temperature, especially night temperature (Bosland & Votava 2000, Aloni et al. 1999, Rylski 1972). Kafizadeh *et al*. (2008) studied the effects of heat on pollen grains in pepper (*C. annuum*) under greenhouse conditions. They found that pollen viability and pollen tube growth was considerably reduced at 38°C as compared with the 25°C. Pollen tube growth inside the style at 38°C was twisted and they grew in spiral and helical forms. Temperatures above 32°C can cause serious pollination and fertilization problems and result in blossom and fruit dropping (Guo et al. 2014). However, when temperature decreases below 15°C, pepper bloom stop (Mercado et al. 1997b).

Rylski and Spigelman (1982) conducted experiment trials on sweet pepper under controlled temperature conditions and natural light. In the first trial, they examined night temperatures of 15, 18, 21 and 24°C (± 1) in combination with a day temperature of 24°C, and in the second trial day temperatures of 22, 25 and 28°C (12 hours) and divided day temperatures of 28-32-28°C (4+4+4 hours) in combination with a night temperature of 18°C. The highest fruit-set was occured at the lowest night temperature. The highest night temperature caused considerable blossom drop, but the highest tested day temperature did not cause increased blossom drop. Low temperatures result in malformed fruits, partly due to absence of seeds and thickening of the style. Similar results were also obtained by several authors (Polowick & Sawhney 1985, Pressman et al. 1998, Pressman et al. 2006, Shaked et al. 2004).

The effects of temperature on yield of sweet pepper (*C. annuum*), in a glasshouse experiment studied by Bakker and van Uffelen (1988). Yields of total and class 1 fruit and number of class 1 fruit showed a maximum at a 24-h mean temperature of 21-21.5°C. Raising the 24-h mean air temperature within the range 16.3 to 23.8°C significantly reduced the mean fruit weight of class 1 fruits. The effect of the day/night temperature amplitude on yield was found of minor importance compared to the effect of 24-h mean temperature.

Sweet pepper (*C. annuum*) shows an irregular yield pattern known as ‘flushing’, where periods of high fruit set and low fruit growth alternate with periods of low fruit set and rapid fruit growth, particularly for large-fruited cultivars (Heuvelink et al. 2004, Sideman 2020). This is considered a significant problem for growers to achieve regular weekly demands during the whole cultivation plants and it results in weeks with a high market supply and low prices alternating with weeks with a low market supply and high prices. Cyclic fluctuations in fruit yield are caused by fluctuations in abortion that could reaches approximately 70% to 80% of the reproductive organs (i.e., buds, flowers, and young fruits) (Wubs *et al*. 2009a). The yield of sweet pepper depends on the fruit set and abortion rate (Wubs *et al*. 2009b). A literature review showed that in peppers there are two theories on possible causes of flower and fruit abortion, with the first is related to the competition between reproductive organs of various ages on the same plant for assimilates (Marcelis *et al.* 2004), while the second concerns hormone flows generated by the fruits (Bangerth, 1989, 2000). In the first theory source and sink strength are used as terms and these refer to the supply of, and demand for, assimilates, respectively. Source strength is the supply of assimilates and takes into account leaf area, radiation, CO_2 level and temperature. Sink strength is the demand for assimilates of the fruits and vegetative parts. Sink strength is the demand for assimilates of the fruits and vegetative parts. Source strength is the supply of assimilates and takes into account leaf area, radiation, CO_2 level planting and density. Assimilate demand of the fruits depends on their number, age, and cultivar.

Photosynthetic radiation (PAR) in the 400–700 nm wavelengths of the visible light spectrum provides the energy required for plant growth and pressure potential required for transpiration (Baxevanou *et al.* 2007). Near infrared radiation (NIR) in the 700–3000 nm wavelengths is converted to sensible and latent long wave heat radiation (LWR) upon entry into the greenhouse. Some of the heat may be lost through the cover but most of the heat is reflected back into the greenhouse resulted in an increase in the internal greenhouse temperature. For most greenhouse crops this increase may be beneficial in cold regions and in the subtropics during winter, but it is considered a significant problem for growers in the sub-tropics in summer time when solar radiation is mostly high (Mashonjowa *et al*. 2010). Among the three aspects of light which are important, duration (photoperiod), quality (i.e., different wavelengths) and intensity, the effect of the last one on fruit abortion has received most attention (Ascough *et al.* 2005).

Sweet pepper has been proven to be well adaptable to a shaded environment (Kitta *et al.* 2014). Shade nets are used in tropical and subtropical areas for sweet pepper production (El-Aidy et al. 1993, Ilic et al. 2011, Rylski & Spigelman 1986). Black nets are most commonly used in sweet pepper. A literature review shows that low light intensity induced flower abortion of pepper crop when shading (40 - 80%) was applied (Wien et al. 1989, Aloni et al. 1994, Shifriss et al. 1994). These results agree with those found by Rylski and Spigelman (1986), where a reduction in radiation during summer increased production in pepper crop, compared with exposure to full sunlight, because of the adverse effect of high temperatures on fruit set. Díaz-Pérez (2013, 2014) study the application of shading levels 0%, 30%, 47%, 62%, and 80% to a sweet pepper crop (*C. annuum*). It was found that high shade levels reduced leaf temperature and excessive leaf transpiration but resulted in reduced leaf photosynthesis. He was further concluded that the moderate shade levels (30% and 47%) were the most favorable for bell pepper plant growth and function. The total marketable yield increased with increasing shade level to a maximum at 35% shade and then decreased with further increments in shade level.

Modification of spectral quality via colored shade nets can act as a physiological tool to modify the crop microenvironment and promote plant growth and yield (Ilic *et al*. 2017). Shading reduced the appearance of pepper cracking and eliminated sunscald on pepper fruits compared to non-shading conditions. Furthermore, studies have shown that colored shade nets induced fruit yield and quality, such as reduction in incidences of physiological disorders (Ombódi et al. 2015, Shahak 2008). Lopez-Marin *et al.* (2012) conducted an experiment to determine the effects of shade among greenhouses that were either not shaded (control treatment) or shaded with aluminized screens of shade levels 40% and 60%. The yield under shade level 40% treatment significantly increased than under control. However, no statistical differences were found between the yields of treatment with shade levels 60% and control treatment.

The literature related to the influence of whitening on greenhouse microclimate and crop response is very limited. Whitening increases diffuse radiation inside the greenhouse (Goudriaan *et al*. 1994). Kittas *et al.* (1999) study the influence of cover material and shading on the spectral distribution of light during summer in soilless rose (*Rosa hybrida*) crop. Whitening applied onto a glass material enhanced slightly the photosynthetically active radiation waveband (PAR) proportion of the incoming solar irradiance, reducing the proportion of infrared radiation. Baille *et al.* (2001) study the microclimate in a Greek glasshouse with a roof vent without whitening and with whitening. The transmissivity of the greenhouse cover decreased from 0.62 without whitening to 0.31 with whitening. It was strongly highlighted the beneficial effect of whitening on both the microclimate and crop behaviour during summer in a warm climate.

The ideal relative humidity (RH) levels for most greenhouse crops range between 60 and 90% (von Zabeltitz 2011). Values above 95% of RH reduce plant transpiration rate and limit nutrient transport to the plant organs. This increases the incidence of physiological disorders such as blossom end rot (BER) in sweet peppers (Gázquez *et al*. 2006). High internal RH can also result in increased incidence of fungal diseases when condensation occurs on the crop canopy (Bailey 2006, Max et al. 2009). On the other hand, values below 60% induce high transpiration rates leading to plant water stress, especially in young crops with low leaf area index (Bailey 2006). Low RH leads to reduced stem lengths and leaf sizes, which inhibit plant growth (Farooq *et al.* 2009). Also, few fungi develop in low RH. It has been observed a reduction in the stomatal conductance of horticultural crops when VPD values are in excess of 1.5 -2 kPa (Jolliet & Baille 2002, Katsoulas et al. 2002). The thresholds of VPD above which both the physiological fluxes and photosynthesis rate are decreased related not only on the plant type and stage of development but also on the prevailing environmental condition to which the plant were cultivated (Medrano *et al.* 2005).

The effects of relative humidity (RH) on fruit set in pepper (*C. annuum*) cv. 'Verbeterde Glas' were investigated in a glasshouse experiment studied by Baër and Smeets (1978). These authors observed that fruit set in pepper does not seem to depend on the RH. They did not find differences in the percentages of fruit abortion at constant RH of 55, 80, or 95%, although the number of fruit set was lower at the highest RH. Furthermore, fewer flowers were formed at the highest RH.

The effects of air humidity on flowering, fruit set, seed set, and fruit growth of glasshouse sweet pepper (*C. annuum*) cv. 'Delphin' studied in a glasshouse experiment, during the early post-planting period from early December until mid-Apri (Bakker 1989). A constant high or low RH and alternating high and low RH by day and night were applied, with the vapour pressure deficit

(VPD) of the glasshouse air varied from 0.33 to 0.66 kPa by day, from 0.27 to 0.86 kPa by night and the 24-h average from 0.30 to 0.75 kPa. Numbers of flowers and fruits were negatively correlated with RH during the night. Furthermore, no significant effect of VPD was found on fruit shape (length/width ratio), number of cavities per fruit, pericarp thickness, dry matter content and fruit maturation rate, while fruit abortion was negatively correlated with RH during the day. Fruit set and number of seeds per fruit were increased by low VPD by day.

The effect of RH on flower production, fruit set, and physiology of bell pepper during elevate temperature and VPD in a glasshouse experiment studied by Erickson and Markhart (2001). The experiment was conducted over two time periods, in three different growth chambers for each experiment, with treatment settings of 25^{o}C with a VPD of 1.1 kPa (60% RH), 33^{o}C with a VPD of 1.1 kPa (75% RH), and 33^{o}C with a VPD of 2.1 kPa (60% RH). They did not observed at high temperature effect of a constantly low RH on the numbers of flower buds and flowers, compared to higher RH. In addition, VPD may have an effect on pepper productivity at lower temperatures, but the stress caused by the high temperature treatment masked any effect of the increased VPD. They mentioned that the high rate of fruit abortion at high temperatures was not due to water stress caused by a low RH.

The influence of greenhouse humidity control on greenhouse microclimate, yield and fruit quality in a summer-to-autumn pepper cultivation studied by Katsoulas *et al.* (2007). Under the use of a fog system, the air VPD was lower than 2 kPa, even during the warmest part of the day, while under no fog conditions it reached values near 4 kPa. Mean fruit weight and the percentage of marketable fruits were positive affected by the fog system, while the total number of fruits per plant significantly reduced. These authors stressed that fruit set probably affected by high levels of RH, mainly when combined with lower levels of incoming solar radiation due to greenhouse shading, which may be explained by the less pollination and higher flower abortion. The marketable fruit yield of sweet pepper did not find to be reduced by fogging, because of microclimate prevailing in greenhouses during summer. The above results do not agree with the observations of Gazquez *et al.* (2006), who studied in three similar greenhouses the yield response of a sweet pepper crop due to the influence of three methods of cooling consists of white washing, fogging and forced ventilation. It was found that the crop subjected to the fog system showed the highest incidence of BER resulted in significantly lower marketable yield.

The use of CO_2 to enrich the atmosphere in greenhouses has been studied since the beginning of the 20th century. The atmospheric CO_2 concentration has increased by the time before the industrial revolution and it is predicted to reach 1000 μmol mol^{-1} by the end of this century (IPCC 2014). Optimal CO_2 concentrations in greenhouses that can promote the yield of vegetables lie between 700 and 1000 μmol mol^{-1} (Gruda and Tanny 2014). Elevated CO_2 has been widely adopted as a gas fertilizer in greenhouse vegetable cultivation, particularly in recent decades as greenhouse technologies have improved (Mortensen 1987, Bisbis et al. 2018)

Dieleman *et al*. (2007) observed that the effect of CO_2 on fruit abortion was apparent throughout the whole cultivation period rather than in the short term where fruit set also related with the number of growing fruit. Nederhoff and Van Uffelen (1988) studied the effect on fruit set and yield of sweet pepper (*C. annuum*) subjected to six CO_2 treatments. They found that fruit set enhanced by 450 μmol mol^{-1} CO_2 level compared to 344 μmol mol^{-1} (ambient) level, partly due to improved photosynthesis. Vegetative growth tended to decrease at higher CO_2 levels, probably because of competition between vegetative and generative organs. Higher fruit yields under elevated CO_2 levels were also observed by Aloni and Karni (2002), and by Dieleman *et al.* (2003). The former authors found significant higher fruit yields when CO_2 concentration was approximately 700 μmol mol^{-1} compared to 350 μmol mol^{-1} (ambient) CO_2 concentration.

Optimizing other environmental factors with CO_2 further increased plant productivity and yield (Kirschbaum 2011). Fierro *et al*. (1994) conducted an experiment to study the response of supplemental CO_2 and light on pepper seedling growth and yield. They found that enrich the atmosphere in greenhouse under CO_2 levels of 900 μmol mol^{-1} with additional light (ambient + 100 μmol m^{-2} s^{-1} photosynthetically active radiation or PAR) significantly increased the early yield of pepper. However, the increases in vegetable yield conferred by CO_2 are decreased to an extent with respect to sweet pepper under drought stress (Rezende *et al.* 2003).

In crops as pepper grown in closed hydroponic systems (CHS), the net volume of supplied water is essentially equal to that removed via transpiration, if the whole amount of collected drainage solution (DS) is consistently recycled. Furthermore, the input ratio between the mass of a nutrient and the volume of water in a CHS is equal to the concentration of this nutrient in the nutrient solution (NS) supplied to the plants to compensate for nutrient and water uptake by plants. This NS, which is mixed with the DS to be recycled, is commonly termed “nutrient solution for closed systems” (NSCS) (de Kreij *et al*. 1999). The unit used to measure nutrient to water uptake ratios is mass of nutrient per water volume, which is identical to that used for concentrations in nutrient solutions. Therefore, several authors use the term “uptake concentration” (UC) to describe the nutrient to water uptake ratios (van Noordwijk 1990, Adams 2002, Sonneveld 2002, Carmassi et al. 2005, Gallardo et al. 2009, Neocleous & Savvas 2015). According to Savvas *et al.* (2017) nutrient-to-water uptake ratios (UC) for N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, and B were estimated by the removal of nutrients from the nutrient solution. In particular, the mean UC of the x macro- or micro-nutrient (C_{xu} in mmol L^{-1} for macro- and μmol L^{-1} for microelements, where x = N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, B) was determined using the following equation:

$$C_{xu} = (V_r (C_{xbi} - C_{xei}) + V_{ui} C_{xa}) / V_{ui}$$

where V_r (L) denotes the total volume of recirculating nutrient solution in each experimental unit, V_{ui} (L) denotes the total volume of nutrient solution consumed by the plants in each experimental unit, C_{xbi} and C_{xei} (μmol L^{-1}) denote the concentrations of the x nutrient in the recirculating nutrient solution on the first and the last day of the i developmental stage, and C_{xa} (mmol L^{-1} or μmol L^{-1}) denotes the concentration of the x macro- or micro-nutrient, respectively, in the replenishment nutrient solution. Total nitrogen UC shall be determined summing over nitrate and ammonium concentrations.

To avoid depletion or accumulation of nutrients in the root zone of a crop grown in a CHS, their concentrations in the NSCS should be equal to the corresponding nutrient to water uptake ratios by the plants. Nutrient uptake and water uptake respond differently to changes in the greenhouse microclimate, since physiologically these are two fully independent processes (Sonneveld & Voogt 1990). However, CO_2 and water vapor share the same transport pathways as they pass through leaf stomata, though to different directions. As a result, the fluctuations in the transpiration to assimilation ratio, as expressed in terms of water use efficiency, are small for a particular plant species (Hopmans & Bristow 2002). Since the rates of carbon fixation determine also the plant dry mass accumulation and concomitantly the plant nutrient requirements (Seginer 2003), the nutrient-to-water uptake ratio exhibits a noticeable stability with time as has been shown by several investigators (Adams & Ho 1993, Savvas & Lenz 1995, Gallardo et al. 2009). This is particularly true if the climatic conditions do not change dramatically compared to optimal levels (Adams 2002, Sonneveld & Voogt 2009, Tzerakis et al. 2013). Nevertheless, major changes in climatic parameters may differently influence the uptake rates of one or more nutrients compared to those of water uptake, thereby imposing commensurate changes in their uptake ratio (Kläring *et al.* 1997). Recent studies indicated that in crops grown hydroponically under hot and dry climatic conditions such as those prevailing in the Mediterranean basin, the UC may be substantially different than those observed in north-European greenhouses (Neocleous & Savvas 2015, Savvas et al. 2017). Standard recommendations for macro- and micro-nutrient levels in NSCS for peppers are mostly based on research carried out in the Netherlands (de Kreij et al. 1999, Sonneveld & Voogt 2009). However, standard recommendations about the composition of a NSCS for a particular plant species should be based on experimental data originating from similar climatic zones. If the mean UC of all essential nutrients supplied via NS to a particular crop species are known, an appropriate NS composition can be established for the NSCS to be supplied to this crop species (Neocleous and Savvas 2015).

Different cultivars, or different rootstocks in the case of grafted plants, may also have an impact on nutrient and water uptake, thereby modifying the UC observed in crops of self-rooted plants (Savvas et al. 2010, Rouphael et al. 2016). Indeed, the uptake of nutrients by grafted plants may be influenced not only by the shoot but also by rootstock genotype (Savvas *et al.* 2017). To the best of my knowledge, in the international scientific literature there is a lack of data about UC arising from experiments with sweet pepper cultivated under hot and dry climatic conditions. Furthermore, grafting proved an efficient technology to increase the tolerance of fruit vegetables to several abiotic stress factors such as salinity, nutrient deficiencies or toxicities, exposure to heavy metals, etc. (Rouphael *et al.* 2017). Grafting is not associated with agrochemical input to the crops and, therefore, it is considered an environment-friendly operation in integrated vegetable production (Rivard & Louws 2008, Schwarz et al. 2010). Therefore, grafting serves in many cases as a rapid alternative to breeding, which is considered a relatively slow methodology of conferring tolerance to biotic and abiotic stress, while its effectiveness is frequently restricted due to a narrow genetic variability (Flores et al. 2010, Colla et al. 2010). Grafting is used as an efficient crop protection technology in fruit vegetable production for more than 50 years in many parts of the world (Bie *et al*. 2017). The application of grafting is less widespread in commercial production of sweet pepper compared to tomato, cucumber and watermelon (Lee *et al.* 2010). Nevertheless, in recent years, several rootstocks used to graft sweet pepper proved to confer considerable tolerance to biotic and abiotic stress factors (Penella et al. 2016, Sánchez-Solana et al. 2016, López-Marín et al. 2017). Therefore, given the high variability of commercial pepper varieties (Tsaballa et al. 2015, Silvar & García-González 2016), the interest in grafting of pepper has increased appreciably among greenhouse pepper producers in recent years (Penella and Calatayad 2018). However, compared to tomato, very little is known about the impact of grafting on nutrient uptake by pepper. Furthermore, to our best knowledge, no report has been published to date in peer-reviewed journals addressing the impact of suboptimal or low temperature on nutrient uptake by grafted pepper plants.

The availability of good-quality water in dry and hot areas is restricted due to low precipitation and high evapotranspiration (El Mahmoudi *et al.* 2011). However, the urban population in the Mediterranean region is expected to increase thereby resulting in increased consumption of water (UN 2014), while at the same time the consequences of global climate change are compromising crop production (Lesk *et al.* 2016). In the Mediterranean soils, hot and dry conditions increase the release rates of Ca ions to the soil solution (Savvas *et al.* 2008). As a result, the Ca concentration in the water resources used to irrigate horticultural crops reaches excessive values, compared to plant needs (Neocleous and Savvas 2013). Considering the fact that also the rapidly developing real estate and the touristic industry in the Mediterranean region increases the water consumption requirements, many growers have no other alternative that to utilize ground water with excessively high concentrations of not only sodium chloride (NaCl) but also Ca. Sweet pepper is generally considered a salt sensitive species (Maas & Hoffman 1997, Sonneveld & van der Burg 1991). A literature review showed that exposure of pepper to high NaCl-salinity can restrict macro- and micronutrient uptake (i.e., K and Ca) and translocation to the leaves (Lycoskoufis et al. 2005, de Kreij 1999, Grattan & Grieve 1999), chlorophyll and carotenoid contents (Colla et al., 2013) and net assimilation rates (Rouphael et al. 2017, Navarro et al. 2002). Excessive salt concentrations in the soil solution and the irrigation water, as well as nutrient imbalances in the root environment, primarily too high K/Ca ratios, can also increase the incidence of calcium-related physiological disorders, such as blossom-end rot (BER) (Grattan & Grieve 1999, Ho and White 2005). The susceptibility of pepper to salinity was found to be cultivar-dependent (Chartzoulakis and Klapaki 2000), as new commercial varieties (hybrid `Orlando') proved to be more sensitive to salinity than older ones (Navarro et al. 2002, Post and Klein-Buitendijk 1996). The soilless culture systems in greenhouses can help not only to decrease the consumption of water because of more accurate irrigation dosing, but also to irrigate using water of marginal quality with respect to salinity without problems for the growing media (Bradley & Marulanda 2000, Montesano et al. 2015). However, in open soilless crops, a considerable fraction of nutrient solution has to drain out of the root zone after each irrigation event to ensure sufficient water supply to all plants (Savvas and Gruda 2018). The fraction of discharged drainage water is influenced by several parameters, but under normal growing conditions, it ranges between 20% and 50%, although this value can increase up to 80% at the beginning of the crop cycle or with low temperatures (Grewal *et al.* 2011). As a result, precious water and fertilizers are wasted, while nitrates can contaminate the groundwater. Therefore, the environmental sustainability of open soilless systems has been questioned (Sonneveld 2002, Gallardo et al. 2009). In order to minimize the environmental impact of greenhouse production, switching over from open to closed soilless systems is suggested (Savvas 2002). A common problem encountered in closed hydroponic systems, especially when the source water is of poor quality, is the accumulation of ions, including non-nutrients which may be toxic (e.g. sodium), but also nutrients contained at excessive concentrations compared to plant requirements, particularly Ca, Mg, SO_4. More specifically, this is the case when the ion to water inlet ratios (i.e. concentrations in the irrigation water) are higher than the corresponding ion to water uptake ratios (Savvas *et al*. 2007).

Taking the above into consideration this dissertation aims to:

a. test the impact of greenhouse temperature and grafting on total yield, water use efficiency and nutrient uptake, on pepper crop grown in a closed hydroponic system (study 1).

b. determine mean UC of macro- and micronutrients during five developmental stages in different pepper cultivars grown in a closed hydroponic system under Mediterranean climatic conditions, by measuring the water uptake and the nutrient removal from the RNS (study 2).

c. determine maximum Ca levels that do not harm the pepper crop grown in a closed hydroponic system and to simulate the pattern of Ca accumulation when the Ca concentration in the irrigation water is excessive (study 3).

The data of the thesis will be introduced to the Nutrisense Software, a novel software for hydroponic crops and especially for closed hydroponic systems, and will be used to establish standard NS compositions for hydroponic cultivation of pepper crop in closed systems under Mediterranean climatic conditions. This software enables proper recycling of the drainage effluents, thereby contributing to considerable savings of water and fertilizers and reduced contamination of water resources by nitrates, something very useful for producers in Mediterranean countries and especially for Greece, as such technologies have so far only been tested in North-Europe (e.g. in The Netherlands).

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CHAPTER 2. EFFECTS OF TEMPERATURE AND GRAFTING ON YIELD, NUTRIENT UPTAKE, AND WATER USE EFFICIENCY OF A HYDROPONIC SWEET PEPPER CROP

2.1 Abstract

In areas characterized by mild winter climate, pepper is frequently cultivated in unheated greenhouses in which the temperature during the winter may drop to suboptimal or even lower levels. Under low temperature (LT) conditions, the uptake of nutrients may be altered in a different manner than that of the water and thus their uptake ratio, known as uptake concentration, may be different than in greenhouses with standard temperature (ST) conditions. In the present study, pepper plants of the cultivars ‘Sammy’ and ‘Orangery’, self-grafted or grafted onto two commercial rootstocks ('Robusto' and 'Terrano'), were cultivated in a greenhouse under either ST or LT temperature conditions. The aim of the study was to test the impact of grafting and greenhouse temperature on total yield, water use efficiency and nutrient uptake. The LT regime reduced yield by about 50% in ‘Sammy’ and 33% in ‘Orangery’, irrespective of the grafting combination. Grafting of ‘Sammy’ onto both 'Robusto' and 'Terrano' increased the total fruit yield by 39% and 34% compared with the self-grafted control, while grafting of ‘Orangery’ increased yield only when the rootstock was ‘Terrano’. The yield increase resulted exclusively from enhancement of the fruit number per plant. Both the water consumption and the water use efficiency were suppressed by the LT regime but the temperature effect interacted with the rootstock/scion combination. The LT increased the uptake concentrations (UC) of K, Ca, Mg, N, and Mn, while it decreased strongly that of P and slightly the UC of Fe, and Zn. The UC of K and Mg were influenced by the rootstock/scion combination but this effect interacted with the temperature regime. In contrast, the Ca, N, and P concentrations were not influenced by the grafting combination. The results of the present study show that the impact of grafting on yield and nutrient uptake in pepper depend not merely on the rootstock genotype but on the rootstock/scion combination.

2.2 Introduction

The concentration of each nutrient in nutrient solutions supplied to plants in closed hydroponic systems should be equal to the corresponding nutrient to water uptake ratio to avoid depletion or accumulation in the root zone (Sonneveld and Voogt 2009). The unit used to measure nutrient to water uptake ratios is mass of nutrient per water volume, which is identical to that used for concentrations in nutrient solutions. Therefore, several authors use the term “uptake concentration” (UC) to describe the nutrient to water uptake ratios (van Noordwijk 1990, Adams 2002, Sonneveld 2002, Carmassi et al. 2005, Gallardo et al. 2009, Neocleous & Savvas 2015). Nutrient uptake and water uptake respond differently to changes in the greenhouse microclimate, since physiologically these are two fully independent processes (Sonneveld and Voogt 1990). However, CO_2 and water vapor share the same transport pathways as they pass through leaf stomata, though to different directions. As a result, the fluctuations in the transpiration to assimilation ratio, as expressed in terms of water use efficiency, are small for a particular plant species (Hopmans & Bristow 2002). Since the rates of carbon fixation determine also the plant dry mass accumulation and concomitantly the plant nutrient requirements (Seginer 2003), the nutrient-to-water uptake ratio exhibits a noticeable stability with time as has been shown by several investigators (Adams & Ho 1993, Savvas & Lenz 1995, Gallardo et al. 2009). This is particularly true if the climatic conditions do not change dramatically compared to optimal levels (Adams 2002, Sonneveld & Voogt 2009). Nevertheless, major changes in climatic parameters may differently influence the uptake rates of one or more nutrients compared to those of water uptake, thereby imposing commensurate changes in their uptake ratio (Kläring *et al*. 1997). This is particularly true if a climatic parameter strongly deviates from the optimal range, thereby imposing stress conditions that alter differently the uptake of a nutrient than that of water, at a physiological level other than transpiration.

The temperature threshold for growth of most of the chilling-sensitive fruit vegetables, such as pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), tomato (*S. lycopersicum* L.) and melon (C. *melo, cucrbita*), is about 8–12 °C (Hansen & Hara 1994, Criddle et al. 1997). In most Mediterranean greenhouses used for production of fruit vegetables, heating during the cold season of the year is either not applied, or applied sporadically, aiming merely to maintain the inside temperature to levels higher than the minimum threshold for growth (Mercado *et al*. 1997). However, under low temperature conditions, plant metabolism, and concomitantly nutrient requirements, may change substantially compared to optimal temperature conditions. Furthermore, the transpiration rates and concomitantly the water uptake alter considerably under low temperature conditions (Elfving et al. 1972, Fennell & Markhart, 1998).

Hence, the nutrient to water uptake ratios may change considerably compared to those determined under optimal temperature conditions, if the mean temperature inside a greenhouse is substantially lower than the standard range.

Grafting is used as an efficient crop protection technology in fruit vegetable production for more than 50 years in many parts of the world (Bie *et al*. 2017). Grafting is not associated with agrochemical input to the crops and, therefore, it is considered an environment-friendly operation in integrated vegetable production (Rivard & Louws 2008, Schwarz et al. 2010). Furthermore, grafting proved an efficient technology to increase the tolerance of fruit vegetables to several abiotic stress factors such as salinity, nutrient deficiencies or toxicities, exposure to heavy metals, etc. (Rouphael *et al*. 2017). Therefore, grafting serves in many cases as a rapid alternative to breeding, which is considered a relatively slow methodology of conferring tolerance to biotic and abiotic stress, while its effectiveness is frequently restricted due to a narrow genetic variability (Flores et al. 2010, Colla et al. 2010).

The application of grafting is less widespread in commercial production of sweet pepper compared to tomato, cucumber and watermelon (Lee *et al*. 2010). Nevertheless, in recent years, several rootstocks used to graft sweet pepper proved to confer considerable tolerance to biotic and abiotic stress factors (Penella et al. 2016, Sánchez-Solana et al. 2016, López-Marín et al. 2017). Therefore, the interest in grafting of pepper has increased appreciably among greenhouse pepper producers in recent years. However, compared to tomato, very little is known about the impact of grafting on nutrient uptake by pepper (Ropokis *et al*. 2018). Furthermore, to our best knowledge, no report has been published to date in peer-reviewed journals addressing the impact of suboptimal or low temperature on nutrient uptake by grafted pepper plants. The impact of grafting and suboptimal temperature on nutrient uptake concentrations is very crucial also for the application of closed soilless systems in pepper cultivation in most Mediterranean greenhouses. In view of this background, the present paper was designed to test whether grafting alters the nutrient to water uptake ratios (i.e. uptake concentrations) in pepper crops grown in unheated or inadequately heated greenhouses under Mediterranean climatic conditions.

2.3 Materials and methods

2.3.1 Plant material and growth conditions

The experiment was carried out in two different compartments of a heated glasshouse at the Agricultural University of Athens (37°58'57.8"N 23°42'14.3"E). During the whole experimental period, the minimum day/night temperature was maintained constantly to 21/16 °C and 12/7 °C in compartments 1 and 2, respectively, corresponding to a standard (ST) and a low temperature (LT) treatment, respectively. In both compartments, two sweet pepper cultivars (*Capsicum annuum* L.) either self-grafted or grafted onto two different rootstocks were grown in recirculating nutrient solution (NS) according to the principles of the Nutrient Film Technique (NFT). The two bell pepper cultivars were one blocky type (‘Orangery’) and one elongated type (‘Sammy’), while the two rootstocks were ‘Robusto’ and ‘Terrano’, both belonging to *Capsicum annuum*. The obtained grafting combinations were ‘Sammy’/‘Sammy’, ‘Robusto’/‘Sammy’, ‘Terrano’/‘Sammy’, ‘Orangery’/‘Orangery’, ‘Robusto’/‘Orangery’, and ‘Terrano’/‘Orangery’. Each treatment was replicated three times and thus 36 experimental hydroponic circuits (EHC) were used. Each EHC comprised one channel, 3.0 m in length, 0.015 m in width, and 0.03 m in height, and consisted of an individual supply tank, a pump, and irrigation pipes as shown in Picture 1.

Picture 1. Experimental hydroponic circuits (EHC) in compartment of a heated glasshouse

Splice grafting was performed when seedlings had developed 3-4 true leaves. The rootstock seeds were sown three days before those of the scion to compensate for the slower growth rate of the former in the hetero-grafting treatments. On November 29, when the grafted seedlings had developed 5-6 true leaves, they were transferred into the 36 EHC.

Each EHC accommodated 9 plants and this arrangement resulted in a plant density of 2.5 plants per m^2, as shown in Picture 2.

Picture 2. Pepper plants into Experimental hydroponic circuits (EHC)

In each channel, the NS was automatically pumped at a rate of 0.4 m^3 h^{-1}, while the total volume of NS recirculating in the closed circuit amounted to 3 L per plant. The level of NS in the supply tank was maintained constant using a floater and a pipe connected to a ‘replenishment tank’, which was positioned above it. Thus, the NS consumed by the plants was compensated by automatic injection of a NS with a composition suitable for pepper cultivation in closed hydroponic systems, henceforth termed SSCS (Savvas & Gruda 2018), which was contained in the replenishment tank. The composition of the SSCS was assumed to be roughly equal to standard uptake concentrations for pepper, as estimated in a previous paper (Ropokis *et al*. 2018). The NS consumed by the plants was counted daily by recording the level difference in the replenishment tank and, thus, the cumulative water consumption could be precisely estimated. Once per day, the pH in the recirculating NS was adjusted to 5.6 by adding appropriate amounts of nitric acid (1 N) or potassium hydroxide (1 N) to the supply tank, based on the actual pH level that was measured using a portable pH-meter. All channels were covered with black-white polyethylene sheets to avoid water evaporation. No drainage water was discharged and losses due to technical failures were negligible. Climatic data during the experiment are shown in Figure 2.1.

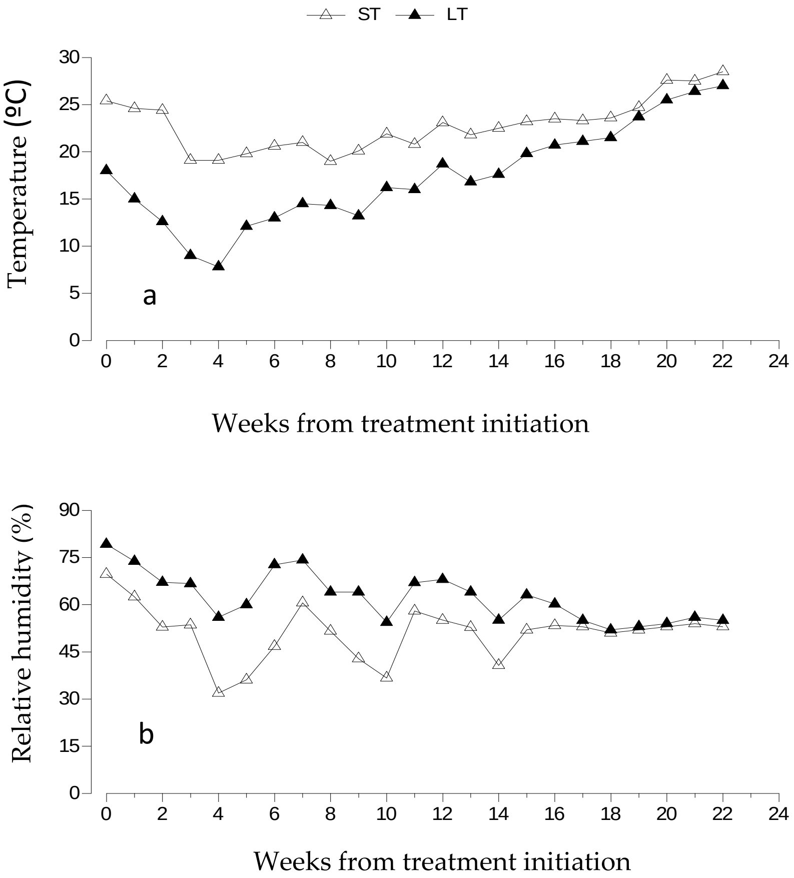

Figure 2.1. Fluctuations of (a) mean temperature (ºC) and (b) mean relative humidity (%) during 790 degree-days from treatment initiation in two greenhouse compartments, in which a standard temperature (ST) and a low temperature (LT) regime were maintained during the experimental period (from 11/29 to 05/20).

Before transplanting, all supply tanks were filled with a starter NS, while all replenishment tanks were filled with SSCS. Immediately after transplanting, SSCS was supplied to the plants through the floater in all circuits, to compensate for nutrient solution uptake by the plants.

2.3.2. Yield, water consumption and water use efficiency

Harvesting commenced on February 6 and on March 23 in the ST and LT compartments, respectively, and terminated on May 20 in both compartments. Commercially ripe fruits were harvested twice per week to estimate the total fresh yield, the total number of fruit per plant and the mean fruit weight in each treatment. Crop water use efficiency was calculated as fruit yield (kg/plant) divided by the cumulative water consumption (L/plant).

2.3.3. Composition and sampling of experimental nutrient solutions

The nutrient concentrations in the starter NS were in all treatments as follows: 6.0 mM K, 6.5 mM Ca, 2.0 mM Mg, 0.5 mM NH_4, 15.6 mM NO_3, 1.2 mM H_2PO_4, 3.1 mM SO_4, 15.0 µM Fe, 10.0 µM Mn, 7.0 µM Zn, 0.7 µM Cu, 60.0 µM B, and 0.5 µM Mo. The EC and pH of the starter NS were 2.6 dS m^{-1} and 5.6, respectively. The nutrient concentrations in the SSCS were as follows: 7.0 mM K, 3.0 mM Ca, 1.25 mM Mg, 1.0 mM NH_4, 12.3 mM NO_3, 1.1 mM H_2PO_4, 1.3 mM SO_4, 15 µM Fe, 10.0 µM Mn, 4.0 µM Zn, 0.7 µM Cu, 25.0 µM B and 0.5 µM Mo.

Immediately after transplanting, i.e. on November 29, samples of starter and replenishment NS were collected from all EHC. After completion of 790 degree-days in both compartments, samples of recirculating NS were collected from all EHC. The collection of NS samples after 790 degree-days coincided with anthesis at the fourth internode in both compartments. However, due to the different temperature regimes in each compartment, the time needed to reach the same degree-days was different. Thus, the samples of recirculating NS were collected on January 10 in the ST compartment, and on February 7 in the LT compartment. Collecting NS samples after completion of the same degree-days rather than the same time interval was aimed at comparing mean uptake concentrations for the same developmental stage, given that plant development is mainly determined by degree-days rather than by time *per se*.

2.3.4. Determination of nutrient concentrations

The concentrations of Ca, Mg, Fe, Mn and Zn in the nutrient solution samples were measured using an atomic absorption spectrophotometer (Perkin Elmer 1100A, Perkin Elmer, Waltham, MA, USA), while that of K was determined by flame photometry (Sherwood Model 410, Cambridge, UK). The concentrations of NO_3-N, NH_4-N, P and B were measured photometrically using a 96-position microplate spectrophotometer (Anthos Zenyth 200; Biochrom, USA). Nitrate N and ammonium N were determined by applying the copperized cadmium reduction method (Griess-Ilosvay procedure) at 540 nm, and the indophenol blue method at 630 nm, respectively. Phosphorus was measured as phosphomolybdate blue complex at 880 nm. Boron was estimated photometrically at 420 nm by the azomethine-H method. The NH_4-N concentration was added to that of NO_3-N in each sample to obtain the total N concentration (Ropokis *et al*. 2018).

2.3.5. Estimation of mean uptake concentrations

Mean nutrient to water uptake ratios (uptake concentrations) for Ca, Mg, K, total-N, P, Fe, Mn, Zn, Cu, and B were estimated by measuring the total removal of each nutrient from the recirculating nutrient solution and the water uptake during a cropping period corresponding to 790 degree-days in both compartments. More specifically, the mean uptake concentration of the i nutrient (C_{iu} in mmol L^{-1} for macronutrients and μmol L^{-1} for micronutrients, where i = Ca, Mg, K, P, total-N, Fe, Mn, Zn and B) was determined using the following mass balance equation (Tzerakis *et al*. 2013):

$$C_{iu} = (V_r\,(C_{ib} - C_{ie}) + V_u C_{ic})/V_u$$

where V_r (L) denotes the total volume of recirculating nutrient solution in each experimental unit, V_u (L) denotes the total volume of NS that was taken up by the plants in each experimental unit during the experimental period, C_{ib} and C_{ie} (mmol L^{-1} for macronutrients and μmol L^{-1} for micronutrients) denote the concentrations of the i nutrient in the recirculating NS on the first and the last day of the experimental period, and C_{ic} (mmol L^{-1} for macronutrients and μmol L^{-1} for micronutrients) denotes the concentration of the i nutrient in the SSCS.

2.3.6. Statistical analysis

The two temperature regimes (NT and LT) and the six scion/rootstock combinations were combined in a 2-factorial split-plot experimental design. The impact of the different treatments was evaluated by applying 2-factorial ANOVA, while multiple comparisons of means were performed by applying the Duncan's Multiple Range Test. The STATISTICA software package, version 12.0 (StatSoft, Inc.,Tulsa, OK, USA) was used to statistically evaluate the data.

2.4 Results

The LT regime reduced the total fruit yield by about 50% in 'Sammy' and 33% in 'Orangery', irrespective of the grafting combination (Table 2.1). Grafting onto both 'Robusto' and 'Terrano' raised the total fruit yield of 'Sammy' by about 35% in comparison to self-grafting. However, when the scion was 'Orangery', only grafting onto 'Terrano' was capable of increasing the total fruit yield compared to self-grafting, whereas grafting onto ‘Robusto’ had no significant impact on yield. The yield increase in ‘Sammy’ due to grafting onto both ‘Robusto’ and ‘Terrano’ resulted exclusively from an increase in the fruit number per plant, while the mean fruit weight was not influenced by grafting. The yield increase imposed by grafting of ‘Orangery onto ‘Terrano’ was also a result of a commensurate increase in the fruit number per plant. Finally, grafting of ‘Orangery’ onto ‘Robusto’ proved to provide no yield benefits not only because it failed to increase the fruit number per plant but also because it reduced significantly the mean fruit weight.

Table 2.1. Impact of temperature (T) and grafting of two cultivated pepper varieties, i.e. ‘Sammy’ (S), and ‘Orangery’ (O), self-grafted or grafted onto the rootstocks ‘Robusto’ or ‘Terrano’ and grown in recirculating nutrient solution on total fruit weight (TFW), total fruit number (TFN) and mean fruit weight (MFW) per plant.

Temperature (°C)	TFW (kg/plant)		TFN (No/plant)		MFW (g)	
Rootstock	S	O	S	O	S	O
Main effects						
Standard T	3.10	2.14	57.10	18.75	54.29	114.13
Low T	1.50	1.43	42.35	20.73	35.42	68.98
Self-grated	1.85 b	1.57 b	42.20 b	16.00 b	43.84	98.13 a
‘Robusto’	2.57 a	1.64 b	53.28 a	20.51 ab	48.24	79.96 b
‘Terrano’	2.49 a	2.14 a	53.70 a	22.71 a	46.37	94.23 a
Statistical significance						
Temperature (T)	***	**	**	ns	***	***
Rootstock (R)	***	*	**	*	ns	*
T × R	ns	ns	ns	ns	ns	ns

ns, *, ** and *** denote “not significant” or significant at $p \leq 0.05$, 0.01 and 0.001 respectively. Means (n=3) followed by different letters within the same column indicate significant differences for each factor according to the Duncan’s multiple range test.

As shown in Table 2.2, the low temperature decreased significantly the water consumption of ‘Sammy’ plants, irrespective of the grafting combination, while it had no significant impact on water consumption of ‘Orangery’ plants.

Table 2.2. Impact of temperature (T) and grafting of two cultivated pepper varieties, i.e. ‘Sammy’ (S), and ‘Orangery’ (O), self-grafted or grafted onto the rootstocks ‘Robusto’ or ‘Terrano’ and grown in recirculating nutrient solution on cumulative water consumption (CWC) and water use efficiency (WUE).

Temperature (°C)	Grafting combination (Rootstock / Scion)	CWC (L plant^{-1})	WUE (g L^{-1})
Standard	‘Sammy’/‘Sammy’	55.00 a	45.95 bc
	‘Robusto’/‘Sammy’	56.31 a	61.62 a
	‘Terrano’/‘ Sammy’	62.72 a	52.54 b
	‘Orangery’/‘Orangery’	35.24 cd	55.71 a
	‘Robusto’/‘Orangery’	36.89 cd	50.74 ab
	‘Terrano’/‘Orangery’	44.70 b	57.71 a
Low	‘Sammy’/‘Sammy’	41.25 bc	28.41 d
	‘Robusto’/‘Sammy’	39.57 bc	42.15 c
	‘Terrano’/‘ Sammy’	37.26 bcd	43.82 c
	‘Orangery’/‘Orangery’	29.73 d	39.59 c
	‘Robusto’/‘Orangery’	34.54 cd	40.66 c
	‘Terrano’/‘Orangery’	38.70 bc	43.99 bc
Main effects			
Standard T		48.48	54.05
Low T		36.84	39.77
	‘Sammy’/‘Sammy’	48.13	37.18
	‘Robusto’/‘Sammy’	47.94	51.89
	‘Terrano’/‘ Sammy’	49.99	48.18
	‘Orangery’/‘Orangery’	32.49	47.65
	‘Robusto’/‘Orangery’	35.72	45.70
	‘Terrano’/‘Orangery’	41.70	50.85
Statistical significance			
Temperature		***	***
Grafting combination		***	**
Temperature × grafting combination		**	**

** and *** denote significant at $p \leq 0.01$ and 0.001 respectively. Mean (n=3) followed by different letters within the same column indicate significant differences for each factor according to the Duncan’s multiple range test.

Grafting onto the rootstock ‘Robusto’ had no impact on plant water consumption, while grafting onto ‘Terrano’ increased the water consumption only when the scion was ‘Orangery’. The water use efficiency (WUE) decreased considerably at low temperature, irrespective of the rootstock/scion combination. Hetero-grafting of ‘Sammy’ increased significantly the WUE irrespective of the scion at LT, but only when the rootstock was ‘Robusto’ at ST. However, grafting of ‘Orangery’ had no impact on WUE, irrespective of the rootstock and the temperature regime. The uptake concentration (UC) of K was significantly higher under LT than under ST conditions in self-grafted plants as well as in plants grafted onto ‘Robusto’, irrespective of the scion (Table 2.3). However, this difference was not significant in plants of both ‘Sammy’ and ‘Orangery’ when they were grafted onto ‘Terrano’ because ‘Terrano’ increased significantly the UC of K by both ‘Orangery’ and ‘Sammy’ under ST conditions compared to the other grafting combinations. The UCs of calcium were not influenced by any grafting combination but was significantly higher at the LT regime in the greenhouse. Similarly to K, the UC of magnesium was significantly higher under LT than under ST conditions in self-grafted plants as well as in plants grafted onto ‘Robusto’, irrespective of the scion, while they were not influenced by LT when both cultivars were grafted onto ‘Terrano’. Grafting onto both ‘Robusto’ and ‘Terrano’ enhanced the Mg UC in the HT regime while under LT conditions only Robusto was capable of increasing the Mg UC.

Table 2.3. Mean uptake concentrations (UC) of potassium (K), calcium (Ca) and magnesium (Mg) in a pepper crop grown in recirculating nutrient solution during 790 degree-days from treatment initiation, as influenced by temperature (T) and grafting of two cultivated varieties, i.e. ‘Orangery’ and ‘Sammy’, either on their roots or onto the rootstocks ‘Robusto’ and ‘Terrano’.

Temperature (ºC)	Grafting combination (Rootstock / Scion)	K UC (mmol L^{-1})	Ca UC (mmol L^{-1})	Mg UC (mmol L^{-1})
Standard	‘Sammy’/‘Sammy’	5.62 cd	3.02	0.73 d
	‘Robusto’/‘Sammy’	5.94 bc	3.08	1.10 bc
	‘Terrano’/‘ Sammy’	6.90 a	2.97	1.05 bc
	‘Orangery’/‘Orangery’	5.12 d	2.51	0.67 d
	‘Robusto’/‘Orangery’	5.77 cd	3.01	0.94 c
	‘Terrano’/‘Orangery’	6.84 ab	2.91	1.04 bc
Low	‘Sammy’/‘Sammy’	7.03 a	3.34	1.17 b
	‘Robusto’/‘Sammy’	7.04 a	3.64	1.40 a
	‘Terrano’/‘ Sammy’	6.65 ab	3.66	1.12 bc
	‘Orangery’/‘Orangery’	6.81 a	3.54	1.10 bc
	‘Robusto’/‘Orangery’	6.69 ab	3.52	1.32 a
	‘Terrano’/‘Orangery’	6.87 ab	3.70	1.03 bc
Main effects				
Standard T		6.03	2.92	0.92
Low T		6.85	3.57	1.19
	‘Sammy’/‘Sammy’	6.33	3.18	0.95
	‘Robusto’/‘Sammy’	6.49	3.36	1.25
	‘Terrano’/‘ Sammy’	6.78	3.32	1.09
	‘Orangery’/‘Orangery’	5.97	3.03	0.89
	‘Robusto’/‘Orangery’	6.23	3.27	1.13
	‘Terrano’/‘Orangery’	6.86	3.31	1.04
Statistical significance				
Temperature		***	***	***
Grafting combination		*	ns	***
Temperature × grafting		**	ns	**

ns, ** and *** denote “not significant” or significant at $p \leq 0.01$ and 0.001 respectively. Mean (n=3) followed by different letters within the same column indicate significant differences for each factor according to the Duncan’s multiple range test.

The UCs of total N was significantly lower while that of P was significantly higher under standard temperature conditions compared to low temperature, irrespective of cultivar and root genotype

(Table 2.4). The rootstock/scion combination had no significant impact on the UC of total N and P and the interaction between temperature regime and grafting was insignificant.

Table 2.4. Mean uptake concentrations (UC) of total nitrogen (total-N) and phosphorus (P) in a pepper crop grown in recirculating nutrient solution during 790 degree-days from treatment initiation, as influenced by temperature (T) and grafting of two cultivated varieties, i.e. 'Orangery' and 'Sammy', either on their roots or onto the rootstocks 'Robusto' and 'Terrano'.

Temperature (°C)	Grafting combination (Rootstock / Scion)	Total-N UC (mmol L^{-1})	P UC (mmol L^{-1})
Main effects			
Standard T		12.14	0.89
Low T		14.72	0.46
	'Sammy'/'Sammy'	13.36	0.64
	'Robusto'/'Sammy'	13.79	0.68
	'Terrano'/' Sammy'	13.19	0.79
	'Orangery'/'Orangery'	13.37	0.58
	'Robusto'/'Orangery'	13.39	0.66
	'Terrano'/'Orangery'	13.48	0.72
Statistical significance			
Temperature		***	***
Grafting combination		ns	ns
Temperature × grafting		ns	ns

ns and *** denote "not significant" or significant at $p \leq 0.001$. Mean (n=3) followed by different letters within the same column indicate significant differences for each factor according to the Duncan's multiple range test.

Under ST conditions, the UCs of Fe and Zn were significantly higher while that of Mn was significantly lower compared to TL, without any significant interaction between temperature regime and rootstock/scion combination (Table 2.5). Furthermore, the grafting combination had no significant impact on the UCs of Fe and Zn. However, the UC of Mn was significantly influenced by both the cultivar and grafting. In particular, 'Orangery' was taking up significantly more Mn per Liter of water absorbed than 'Sammy', irrespective of the root genotype. Furthermore, hetero-grafting onto both 'Robusto', and 'Terrano' reduced the mass of Mn uptake per L of water absorbed by 'Orangery', while it had no impact on the UC of Mn by 'Sammy'. The UC of B was not influenced by temperature regime or by the rootstock/scion combination.

Table 2.5. Mean uptake concentrations (UC) of iron (Fe), manganese (Mn), zinc (Zn) and boron (B) in a pepper crop grown in recirculating nutrient solution during 790 degree-days from treatment initiation, as influenced by temperature (T) and grafting of two cultivated varieties, i.e. 'Orangery' and 'Sammy', either on their roots or onto the rootstocks 'Robusto' and 'Terrano'.

Temperature (ºC)	Grafting combination (Rootstock / Scion)	Fe UC (μmol L^{-1})	Mn UC (μmol L^{-1})	Zn UC (μmol L^{-1})	B UC (μmol L^{-1})
Main effects					
Standard T		18.46	11.08	7.37	28.97
Low T		17.16	12.06	6.61	29.54
	'Sammy'/'Sammy'	17.80	11.35 c	6.99	29.76
	'Robusto'/'Sammy'	18.09	11.31 c	7.18	30.36
	'Terrano'/' Sammy'	16.93	11.30 c	6.50	28.39
	'Orangery'/'Orangery'	19.04	12.23 a	7.69	29.47
	'Robusto'/'Orangery'	18.34	11.85 b	7.25	29.65
	'Terrano'/'Orangery'	16.66	11.67 b	6.34	28.79
Statistical significance					
Temperature		*	***	*	ns
Grafting combination		ns	***	ns	ns
Temperature × grafting		ns	ns	ns	ns

ns, * and *** denote "not significant" or significant at $p \leq 0.05$ and 0.001. Mean (n=3) followed by different letters within the same column indicate significant differences for each factor according to the Duncan's multiple range test.

2.5 Discussion

Pepper is a warm-season crop with a high susceptibility to suboptimal temperatures (Airaki *et al.* 2012), and hence the yield decreases imposed by low temperature in the present study were fully anticipated. However, in the present study, the suppressive effect of low temperatures on pepper yield did not interact with grafting and the rootstock, which indicates that the tested rootstocks have no impact on cold tolerance of pepper. Shu *et al.* (2016) grafted two pepper cultivars onto five different non-commercial rootstocks and found that only one out of the ten tested grafting combinations had a higher resistance to low temperature. This finding indicates that the tolerance to low temperature in pepper depends not only on the rootstock genotype but also on the genotype of the scion.

The results of the present study showed that grafting can provide significant benefits to pepper crops in terms of total fruit yield, in agreement with findings of previous investigators (Colla et al. 2008, López-Marín et al. 2013, Penella et al. 2016). However, the impact of grafting on fruit yield is depending on the rootstock/scion combination, as also suggested by Rouphael *et al.* (2017), Guimarães *et al.* (2009) and Savvas *et al.* (2010). Indeed, in the current study, both 'Robusto' and 'Terrano' increased the total fruit yield by 39% and 34% compared with the self-grafted control when the scion was the commercial variety ‘Sammy’. However, when the scion was ‘Orangery’, only ‘Terrano’ was capable of increasing the total fruit yield by 35% compared with that obtained from self-grafted ‘Orangery’ plants, while ‘Robusto’ had no impact on yield. The yield increases achieved by grafting pepper plants in the present study are appreciably higher than that reported by Jang *et al.* (2012), which amounted to only 9.2%. In agreement with the results of the present study, López-Marín *et al.* (2013) found that grafting of pepper cv. ‘Herminio’ increased yield only when the rootstock was ‘Creonte’, while grafting of ‘Herminio’ onto ‘Atlante’ and ‘Terrano’ had no impact on yield. These findings show that research on the agronomic impact of grafting in pepper crops should be based on testing each individual rootstock/scion combination rather than on screening different rootstocks using just one scion. López-Marín *et al.* (2013) also found that grafting the sweet pepper cultivar ‘Herminio’onto the commercial rootstock ‘Creonte’ had no significantly impact on leaf biomass but increased the total and marketable fruit yield by 30% and 50% under non-shaded and shaded conditions compared to the non-grafted plants, respectively.

In ‘Sammy’, the absence of any impact of grafting on total plant biomass and water consumption despite the significant increase in the fruit number per plant points to a positive effect of both tested rootstocks on fruit setting rather than on plant vigor. A similar response was observed also by López-Marín *et al.* (2013), with the sweet pepper cultivar ‘Herminio’grafted onto the commercial rootstock ‘Creonte’. On the other hand, when ‘Orangery was grafted onto ‘Terrano’ in the present study, both the plant biomass and the water consumption increased proportionally to the increase in fruit number per plant, which indicates that this grafting combination increases yield due to stimulation of plant vigor. Nevertheless, all grafting combinations increased yield exclusively by raising the number of fruit per plant in the present study, while they had no effect on the mean fruit weight. This was surprising, as in a previous study, grafting of pepper was found to affect the fruit shape (Tsaballa *et al*. 2013), which means that grafting may have an impact on morphological characteristics of each individual fruit. Nevertheless, Colla *et al.* (2008), who grafted the sweet pepper cultivar Edo onto five different rootstocks, found that four of them increased significantly the total fruit yield and in all cases the yield increase was exclusively due to a higher fruit number per plant.

By definition, the impact of grafting on water use efficiency (WUE) depends on both fruit yield and water consumption. Thus, sometimes rootstock/scion combinations imposing quite different effects on yield than on water consumption may result in similar effects on WUE. Indeed, grafting ‘Orangery’ onto ‘Robusto’ had no impact on WUE because it had no impact on both fruit yield and water consumption. However, grafting ‘Orangery’ onto ‘Terrano’ had no impact on WUE because this rootstock/scion combination increased proportionally both the fruit yield and the water consumption as it enhanced the vegetative biomass production. Furthermore, grafting ‘Sammy’ increased the WUE irrespective of the tested rootstocks because both ‘Robusto’ and ‘Terrano’ increased yield without affecting the vegetative plant biomass and concomitantly the water consumption via transpiration. Colla *et al*. (2008) and López-Marí*n et al.* (2013) also found that grafting different scions onto the same rootstock and vice versa may result in different yield responses. These results indicate that the impact of grafting on water use efficiency (WUE) depends on the rootstock/scion combination rather than merely on the rootstock or on the scion.

Different rootstock/scion combinations may result in increased yield due to a more favorable modulation of several, metabolic, physiological functions and transcriptomic responses (Ntatsi et al. 2013, Ntatsi et al. 2017). These include favorable changes in hormonal balance between root and shoot (Albacete et al. 2009, Lee et al. 2010, Ntatsi et al. 2014a, Ntatsi et al. 2014b, Venema et al. 2017), improved resistance to recurrent abiotic and biotic stress (Rouphael et al. 2017, Cohen et al. 2017), and enhanced nutrient and water uptake due to a more vigorous root system (Lee et al. 2010, King et al. 2010). In the present investigation, the hormonal balance was not studied, while the single abiotic stress tested, i.e. low temperature, was not alleviated by any grafting combination. However, the measurements of plant biomass production, water consumption and nutrient uptake allows for an assessment of the contribution of nutrient and water uptake to yield enhancement. Thus, the stimulatory effect of grafting on fruit yield under ST conditions in the present study might be partly associated with enhanced K and Mg uptake (for ‘Terrano’) or Mg uptake (for ‘Robusto’). On the other hand, the enhancement of yield by some grafting combinations were observed under both temperature regimes tested in the current study, while the increases in K and Mg uptake concentrations were observed only in plants cultivated under standard temperature conditions. Furthermore, the uptake concentrations of Ca, N, P, and micronutrients were not enhanced by any grafting combination. Thus, a favorable modulation of nutrient uptake efficiency cannot be considered the main reason for the yield increases imposed by some grafting combinations in the present study. Further research is needed to unravel the physiological and molecular mechanisms implicated in the enhancement of total fruit yield by some rootstock/scion combinations.

As reported by Savvas *et al*. (2010) and Rouphael *et al.* (2017), certain grafting combinations may restrict the uptake of some micronutrients and non-nutrient ions, an effect that may be helpful to crops exposed to excessive concentrations of these ions in the root zone. In the present study, both ‘Robusto’ and ‘Terrano’ reduced slightly, but significantly, the Mn uptake by ‘Orangery’, while the same rootstocks had no impact on Mn uptake when the scion was ‘Sammy’. In a previous study, Ropokis *et al.* (2018) also found no impact of grafting on Mn uptake concentrations. The different responses of ‘Sammy’ and ‘Orangery’ to grafting concerning the Mn uptake further support the notion that the impact of grafting on nutrient uptake depends on the specific rootstock/scion combination rather than on the rootstock genotype.

The increases in the uptake concentrations of K, Ca, Mg and N under low temperature conditions was a consequence of the reduction in water consumption by 24%. Especially the increases in the uptake concentrations of Ca (22%) and Mg (27%) were very similar to the reduction in water consumption, which indicates that the absolute amounts of Ca and Mg absorbed by the crop were not restricted by low temperature, despite the reduction in plant biomass. Unlike the uptake concentrations of metallic micronutrient cations, that of P was reduced by low temperature. These results indicate that the uptake mechanism of $H_2PO_4^-$ by pepper is much more susceptible to low temperature than that of K, Ca, Mg and N. As shown by Adams (1994), the uptake of P responds much stronger to the root temperature than the uptake of N and K. The low temperature treatments in the present study resulted in low temperatures also in the root zone, which recovered slower during the day than the air temperature (data not shown). Phosphorus occurs at much lower concentrations in the soil solution compared to other macronutrients (Bieleski 1973). Therefore, during their evolution, plants developed an active uptake mechanism for P uptake, which is much more depending on the availability of metabolic energy in the roots than for uptake of other nutrients (Schachtman *et al*. 1998). However, the low root temperature decreases root respiration and consequently the availability of metabolic energy for active P uptake (Sutton 1969*)*. Thus, the profound reduction of P uptake by pepper in the LT regime may be ascribed mainly to the low root temperature. From another point of view, the results of the present study provide support to the notion that the uptake of P by pepper is stronger influenced by root temperature than by that prevailing in the air.

The UCs found by Voogt and Sonneveld (1997) in a pepper crop grown in a closed rockwool system in the Netherlands, averaged for the whole cropping period, were 2.2 mmol L^{-1} for Ca, 0.78 mmol L^{-1} for Mg, 4.6 mmol L^{-1} for K, 10.30 mmol L^{-1} for N, and 0.81 mmol L^{-1} for P. The UC of macronutrients estimated by Ropokis *et al.* (2018) ranged from 2.4 to 3.7 mmol L^{-1} for Ca, 1.0 to 1.5 mmol L^{-1} for Mg, 6.2 to 9.0 mmol L^{-1} for K, 11.7 to 13.7 mmol L^{-1} for N, and 0.7 to 1.1 mmol L^{-1} for P. Finally, the average UCs found in the present study during the vegetative cropping period under ST conditions were 2.92 mmol L^{-1} for Ca, 0.92 mmol L^{-1} for Mg, 6.03 mmol L^{-1} for K, 12.14 mmol L^{-1} for N, and 0.89 mmol L^{-1} for P. Comparing the data from these three different crops shows that the UC of Ca, Mg, K, and N are generally higher in winter crops of pepper grown under Mediterranean climatic conditions than those reported for Dutch greenhouses, while that of P is similar in both environments. The values found in the present study are comparable with those found by Ropokis *et al.* (2018) in a previous study, although Mg and K tended to be somewhat lower in the current study. Both the present study and that of Ropokis *et al.* (2018) showed that the temperature inside the greenhouse, the cultivar, and the rootstock when using grafted plants, may have a significant impact on the UCs. Since these factors may vary in different crops, the UCs found in the three studies are only indicative. Therefore, their use as a basis to prepare nutrient solutions for closed hydroponic systems entails frequent readjustment following measurements of the nutrient concentrations in the root zone of the crop, as suggested by Savvas and Gruda (2018).

2.6 Conclusion

Minimizing greenhouse heating under Mediterranean climatic conditions so as to maintain the minimum inside day/night temperature at 12/7 °C, respectively, suppressed the fruit yield of pepper to levels depending on the cultivar, but not on grafting onto 'Robusto' or 'Terrano'. The yield decrease imposed by low temperature (LT) resulted from a reduction of the mean fruit weight (MFW) in ‘Orangery’ and from a reduction of both fruit number per plant (TFN) and MFW in ‘Sammy’. Grafting of ‘Sammy’ onto both 'Robusto' and 'Terrano' increased the fruit yield, while grafting of ‘Orangery’ increased yield only when the rootstock was ‘Terrano’. The yield increase imposed by grafting resulted exclusively from enhancement of the fruit number per plant (TFN).
The water use efficiency (WUE) decreased considerably at LT, irrespective of the rootstock/scion combination. Grafting of ‘Sammy’ increased significantly the WUE irrespective of the scion at LT, but only when the rootstock was ‘Robusto’ at ST. Grafting of ‘Orangery’ had no impact on WUE,

irrespective of the rootstock and the temperature regime. The LT increased the uptake concentrations (UC) of K, Ca, Mg, N, and Mn, while it decreased strongly that of P and slightly those of Fe and Zn. The UC of K and Mg were influenced by the rootstock/scion combination but this effect interacted with the temperature regime. In contrast, the Ca, N, and P concentrations were not influenced by the grafting combination. The results of the present study show that the impact the impact of grafting on yield and nutrient uptake in pepper depend not merely on the rootstock genotype but on the rootstock/scion combination.

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CHAPTER 3. IMPACT OF CULTIVAR TYPE AND GRAFTING ON NUTRIENT AND WATER UPTAKE BY SWEET PEPPER (*Capsicum annum* L.) GROWN HYDROPONICALLY UNDER MEDITERRANEAN CLIMATIC CONDITIONS

3.1 Abstract

In closed-cycle hydroponic systems (CHS), nutrients and water should be delivered to the plants at identical ratios to those they are removed via plant uptake, to avoid their depletion or accumulation in the root zone. For a particular plant species and developmental stage, the nutrient to water uptake ratios, henceforth termed "uptake concentrations" (UC), remain relatively constant over time under similar climatic conditions. Thus, the nutrient to water uptake ratios can be used as nutrient concentrations in the nutrient solution (NS) supplied to CHS to compensate for nutrient and water uptake by plants. In the present study, mean UC of macro- and micronutrients were determined during five developmental stages in different pepper cultivars grown in a closed hydroponic system by measuring the water uptake and the nutrient removal from the recirculating NS. The experiment was conducted in a Mediterranean environment and the tested cultivars were 'Orangery', 'Bellisa', 'Sondela', 'Sammy' self-grafted and 'Sammy' grafted onto the commercial rootstock `RS10' (*Capsicum annuum*). 'Sondela' exhibited significantly higher NO_3, Mg, Ca and B UC in comparison with all other cultivars, while Bellisa exhibited higher K UC. The UC of all nutrients were similar in the grafted and the non-grafted 'Sammy' plants, which indicates that this Capsicum annum rootstock does not modify the uptake of nutrients and water by the scion. The UC of macronutrients estimated in the present study (mmol L^{-1}) ranged from 2.4 to 3.7 for Ca, 1.0 to 1.5 for Mg, 6.2 to 9.0 for K, 11.7 to 13.7 for N, and 0.7 to 1.1 for P. The UC of N, K, Ca and Mg were appreciably higher than the corresponding values found under Dutch climatic conditions, while that of P was similar in both environments during the vegetative stage and higher thereafter. The UC of Fe, Zn and B tended to decrease with time, while that of Mn increased initially and subsequently decreased slightly during the reproductive developmental stage.

3.2 Introduction

In crops grown in closed hydroponic systems (CHS), the net volume of supplied water is essentially equal to that removed via transpiration, if the whole amount of collected drainage solution (DS) is consistently recycled. Furthermore, the input ratio between the mass of a nutrient and the volume of water in a CHS is equal to the concentration of this nutrient in the nutrient solution (NS) supplied to the plants to compensate for nutrient and water uptake by plants. This NS, which is mixed with the DS to be recycled, is commonly termed “nutrient solution for closed systems” (NSCS) (de Kreij *et al*. 1999). To avoid depletion or accumulation of nutrients in the root zone of a crop grown in a CHS, their concentrations in the NSCS should be equal to the corresponding nutrient to water uptake ratios by the plants, henceforth termed “uptake concentrations” (UC). For a particular plant species and developmental stage, the UC exhibit an appreciable stability over time under similar climatic conditions (Savvas & Lenz 1995, Sonneveld & Voogt 2009, Tzerakis et al. 2013). Thus, if the mean UC of all essential nutrients supplied via NS to a particular crop species are known, an appropriate NS composition can be established for the NSCS to be supplied to this crop species (Neocleous and Savvas 2015).

Standard recommendations for macro- and micro-nutrient levels in NSCS for peppers are mostly based on research carried out in the Netherlands (de Kreij et al. 1999, Sonneveld & Voogt 2009). However, standard recommendations about the composition of a NSCS for a particular plant species should be based on experimental data originating from similar climatic zones. Indeed, previous research showed that, in crops grown hydroponically under hot and dry climatic conditions such as those prevailing in the Mediterranean basin, the UC may be substantially different than those observed in north-European greenhouses (Neocleous & Savvas 2015, Savvas et al. 2017). Furthermore, different cultivars, or different rootstocks in the case of grafted plants, may have an impact on nutrient and water uptake, thereby modifying the UC observed in crops of self-rooted plants (Savvas et al. 2010, Rouphael et al. 2016). Indeed, the uptake of nutrients by grafted plants may be influenced not only by the shoot but also by rootstock genotype (Savvas *et al*. 2017). To date, in the international scientific literature there is a lack of data about UC arising from experiments with sweet pepper cultivated under hot and dry climatic conditions. Taking this gap of knowledge into consideration, the present study was designed to estimate mean UC for most macro- and micronutrients in a pepper crop grown in a Mediterranean environment, and compare them with similar data arising from north-European climatic conditions. Furthermore, given the high variability of commercial pepper varieties (Tsaballa et al. 2015, Silvar & García-González 2016), as well as the increasing use of grafted seedlings to establish greenhouse crops of pepper

(Penella and Calatayad 2018), UC were established for four different cultivar types of pepper, one of which was either self-grafted or grafted onto a commercial rootstock. The anticipated data can be used to optimize the composition of NSCS supplied to pepper crops grown in CHS under climatic conditions characterized by mild winters, and dry and hot summers.

3.3 Materials and methods

3.3.1 Plant material and growth conditions

The experiment was conducted in a glasshouse at the Agricultural University of Athens (AUA). Four pepper cultivars (*Capsicum annuum* L.) were grown in recirculating nutrient solution according to the principles of the Nutrient Film Technique (NFT). Two cultivars of the bell type (Orangery, Sondela) and two of the elongated form (Bellisa, Sammy), one of which (Sammy) was either self-grafted or grafted on a rootstock RS10 (*Capsicum annuum*). The three non-grafted cultivars and the two versions of 'Sammy' i.e. grafted and non-grafted, constituted five experimental treatments. On 16 January 2014, the plants were transferred into 20 closed-loop hydroponic circuits (experimental plots), which were supplied with nutrient solution as shown in Picture 3.

Picture 3. Experimental hydroponic circuits in compartment of a heated glasshouse

Each circuit comprised one channel, 3.0 m in length, 0.015 m in width, and 0.03 m in height, which. All channels accommodated 9 plants into black–white polyethylene sheets to avoid water evaporation as shown in Picture 4.

Picture 4. Pepper plants into black–white polyethylene sheet of channel.

All plants were supplied with a standard nutrient solution. Each treatment was replicated four times and thus 20 experimental hydroponic circuits were used. In each experimental unit, each of the 20 experimental units consisted of an individual supply tank, a pump, and irrigation pipes, thereby formed a closed circuit in which a nutrient solution (NS) was constantly recirculating according to the principles of the nutrient film technique. Climatic data during the experiment are given in Table 3.1.

Table 3.1. Monthly averages of temperature (ºC) and relative humidity (%) inside the experimental greenhouse.

		January	February	March	April	May	June	July
Temperature (ºC)	Average	21.5	22.5	23.8	27.7	29.3	31.3	34.5
	Min	19.0	19.0	19.2	19.3	19.0	19.5	19.5
	Max	23.4	27.0	32.0	34.0	35.5	37.4	39.4
Relative humidity (%)	Average	72.1	71.6	70.6	68.7	62.5	59.1	52.4
	Min	66.3	63.4	58.4	56.8	48.5	44.4	38.8
	Max	74.4	73.6	72.3	70.6	67.8	63.8	56.6

The nutrient solution was automatically pumped at a rate of 0.4 m^3 h^{-1} to each channel while the total volume of the NS that was recirculating in each experimental unit amounted to 3 L per plant.

In each unit, the nutrient and water uptake were compensated by automatically supplying a replenishment NS from an individual tank using a floater to maintain a constant NS level in the supply tank. The NS consumed by the plants was counted daily by recording the level difference in the tank filled with replenishment NS. The pH in the recirculating NS was adjusted once per day to 5.6 by adding appropriate amounts of nitric acid to the supply tank based on the actual pH level which was measured using a portable pH-meter. All channels were covered with black-white polyethylene sheets to avoid water evaporation. Each channel accommodated 9 pepper plants, while the total volume of recirculating NS in each experimental unit (including both the solution contained in the supply tank and that flowing in the gullies) amounted to 70 L. The plant density was 2 plants per m^2.

The concentrations of all other nutrients in the five treatments were as follows: 6.0 mM K, 6.5 mM Ca, 2.0 mM Mg, 0.5 mM NH_4, 15.6 mM NO_3, 1.2 mM H_2PO_4, 6.5 mM SO_4, 15.0 μM Fe, 10.0 μM Mn, 7.0 μM Zn, 0.8 μM Cu, 50.0 μM B, and 0.5 μM Mo. The EC and pH in the above NS were 2.60 dS m^{-1} and 5.6, respectively. After transplanting, the nutrients and water that were absorbed by the plants were replenished daily by supplying replenishment NS with different nutrient concentrations than in the initial NS. Every four weeks the recirculating NS was discharged and replaced by fresh nutrient solution of the following composition: 6.0 mM K, 5.30 mM Ca, 1.65 mM Mg, 0.5 mM NH_4, 14.6 mM NO_3, 1.2 mM H_2PO_4, 2.0 mM SO_4, 20.0 μM Fe, 12.0 μM Mn, 6.0 μM Zn, 0.8 μM Cu, 45.0 μM B and 0.5 μM Mo. The NS that was consumed by the plants was recorded daily and replaced by refilling the replenishment tank with a nutrient solution of the following composition: Vegetative stage: 5.30 mM K, 3.15 mM Ca, 1.3 mM Mg, 1.4 mM NH_4, 11.6 mM NO_3, 1.1 mM H_2PO_4, 1.2 mM SO_4, 15.0 μM Fe, 10.0 μM Mn, 4.0 μM Zn, 0.8 μM Cu, 30.0 μM B and 0.5 μM Mo; Reproductive stage: 6.0 mM K, 2.7 mM Ca, 1.1 mM Mg, 0.8 mM NH_4, 10.6 mM NO_3, 1.1 mM H_2PO_4, 1.1 mM SO_4, 15.0 μM Fe, 10.0 μM Mn, 0.7 μM Cu, 5.0 μM Zn, 25.0 μM B and 0.5 μM Mo. The pH in the recirculating nutrient solution was daily adjusted to 5.6-5.7 by adding appropriate amounts of 1 N HNO3 stock solution based on the actual pH level which was measured using a portable pH-meter.

3.3.2 Nutrient uptake calculations

Samples of recirculating NS were selected on a 4-weekly basis from all experimental units to determine the actual Ca, Mg, K, P, B, NO_{3-}, Fe, Mn and Zn concentrations. The concentrations of

Ca, Mg, Fe, Mn and Zn in both the nutrient solutions and the aqueous extracts of plant tissues were measured using an atomic absorption spectrophotometer (Perkin Elmer 1100A, Perkin Elmer, Waltham, MA, USA) while K was determined by flame photometry (Sherwood Model 410, Cambridge, UK). The NO_3, P and B concentrations in nutrient solution samples were measured by UV/VIS spectroscopy at 540, 880 and 420 nm, respectively.

Nutrient to water uptake ratios (uptake concentrations) for K, Mg, Ca and Fe were estimated based on the removal of nutrients from the nutrient solution. In particular, the mean uptake concentration of the x micronutrient (C_{xu} in μmol L^{-1}, where x = Ca, Mg, K, P, B, NO_3, Fe, Mn and Zn) was determined for two time intervals using the following mass balance equation (Tzerakis *et al*. 2013):

$$\boldsymbol{C}\mathbf{xu} = \frac{\boldsymbol{Vr\ (Cxbi - Cxei) + VuiCxa}}{\boldsymbol{Vui}}$$

where V_r (L) denotes the total volume of the recirculating nutrient solution in each experimental unit (70 L in the present study), V_{ui} (L) denotes the total volume of NS that was taken up by the plants in each experimental unit during the i time interval (i = 1...3), C_{xbi} and C_{xei} (μmol L^{-1}) denote the concentrations of the x nutrient in the recirculating nutrient solution on the first and the last day of the i time interval (i = 1...3), and C_{xa} (μmol L^{-1}) denotes the concentration of the x nutrient in the replenishment NS used in each treatment.

3.3.3 Statistical analysis

All data were statistically analyzed by applying ANOVA using the PlotIT3.2® software package, version 3.2 for Windows (Scientific Programming Enterprises, Haslett, MI, USA). Data are presented in graphs as means ± SE.

3.4 Results

As shown in Figure 3.1, 'Bellisa' and 'Sondela' consumed about 15% less water than 'Orangery' and 'Sammy' self-grafted, and about 20% less than Sammy grafted onto the commercial rootstock RS10. These differences reflected commensurate differences in the leaf area and the vegetative plant biomass (data not shown).

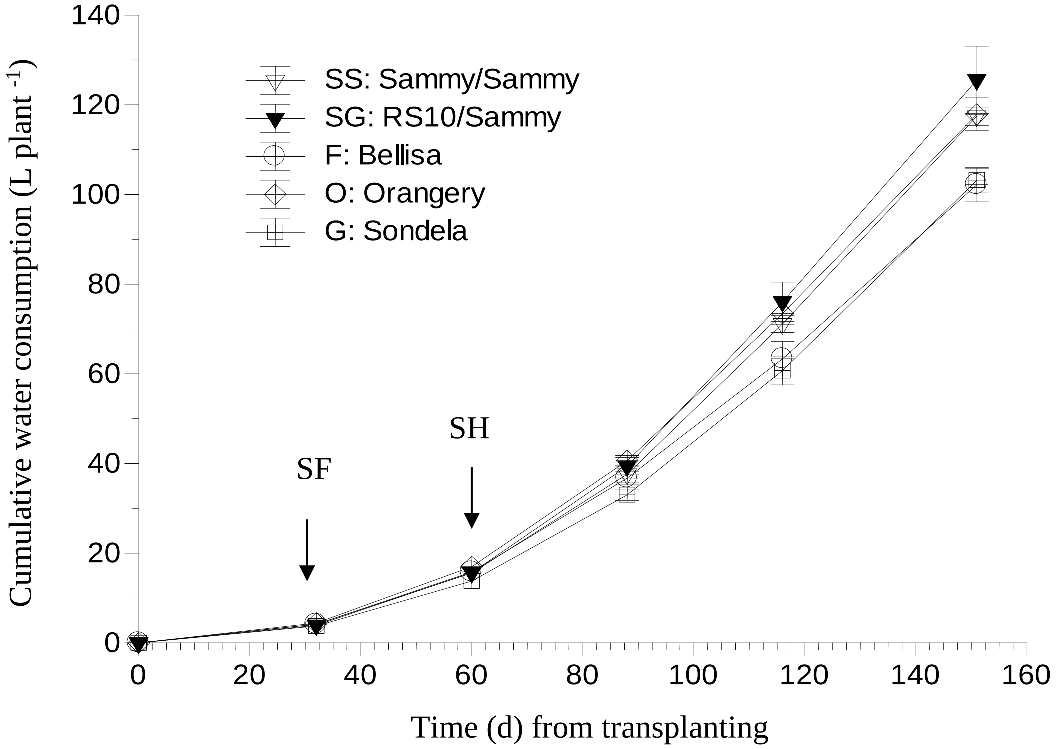

Figure 3.1. Evolution of the cumulative water consumption (CWC) in a pepper crop grown in a closed hydroponic system as influenced by four different pepper cultivars ('Orangery', 'Bellisa', 'Sondela', 'Sammy') and grafting ('Sammy' self-grafted or grafted onto the commercial rootstock `RS10'). Vertical bars indicate ± standard errors of means. SF: start of flowering; SH: start of harvesting.

However, as shown in Figure 3.2, the differences in water consumption and vegetative plant biomass had no significant impact on the total fruit production, although 'Orangery' rendered a slightly higher yield than the other cultivars. In contrast to the total fruit yield, the number of fruit per plant and the mean fruit weight were strongly influenced by the genotype. In particular, 'Orangery' and 'Sondela' produced much less fruit than 'Sammy' but the weight of fruit from the latter cultivar was much smaller. As a result, the total yield was similar in all cultivars without any significant differences between them. On the other hand, hetero-grafting of 'Sammy' onto the commercial rootstock RS10 had no impact on the total fruit yield, the number of fruit per plant or the mean fruit weight.

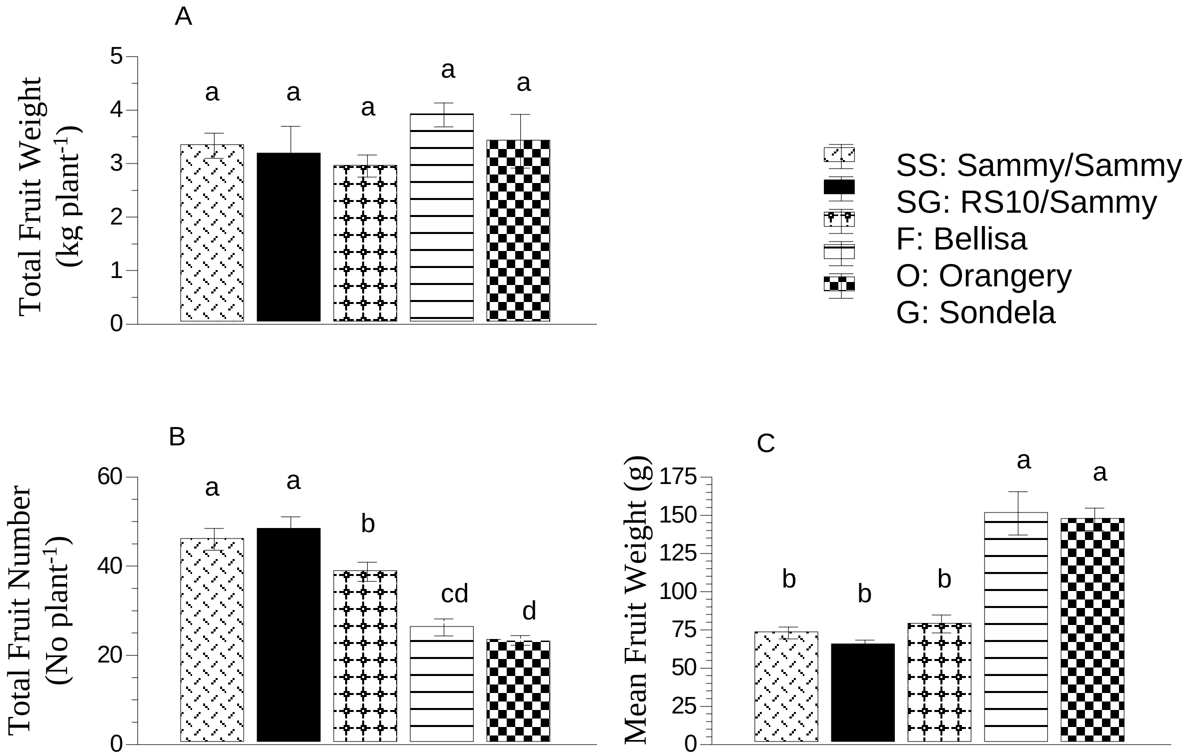

Figure 3.2. Influence of four cultivated varieties. i.e. 'Orangery' (O), 'Bellisa' (F), 'Sondela' (G) and 'Sammy' self-grafted (SS) or 'Sammy' grafted onto a commercial rootstock `RS10' (SG), grown in closed hydroponic systems, on (A) total fruit weigh (TFW) per plant, (B) total fruit number (TFN) per plant and (C) average marketable fruit weigh (MFW) per plant. Vertical bars indicate ± standard errors of means.

Due to the similar yield performance and the significantly lower cumulative water consumption in comparison to 'Sammy' and 'Belisa', 'Sondela' exhibited a significantly higher water use efficiency (WUE) than these two cultivars (Figure 3.3). However, the WUE of 'Orangery' was as high as that of 'Sondela'. It is worth to mention that grafting onto the commercial *Capsicum annuum* rootstock 'RS10' did not improve the WUE of 'Sammy'.

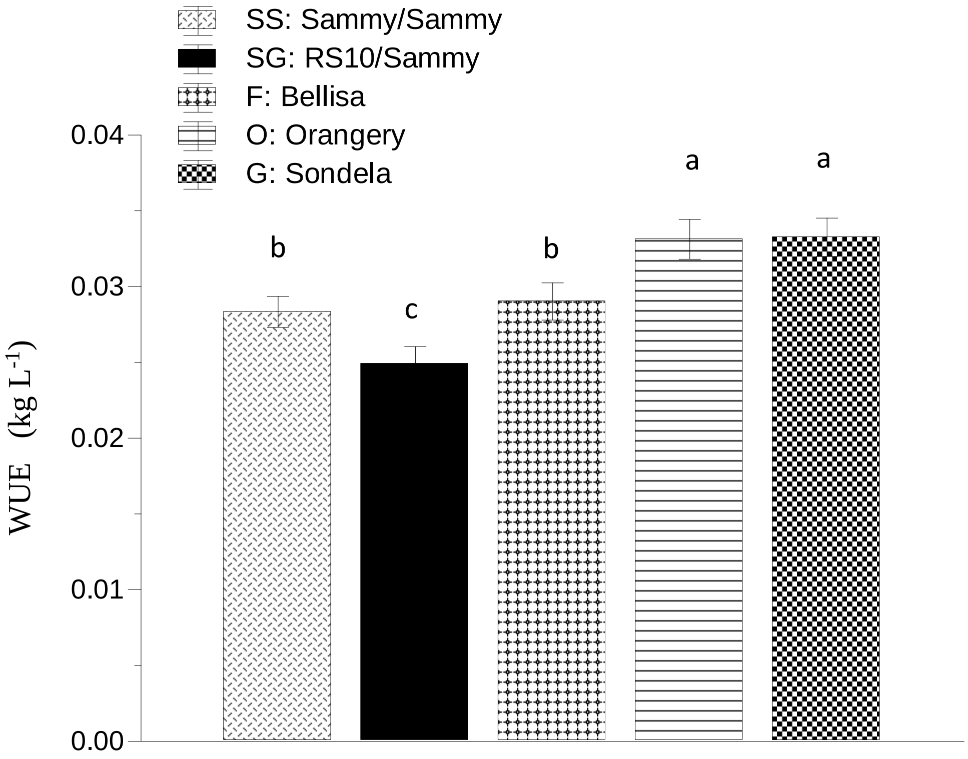

Figure 3.3. Estimated water use efficiency (WUE) in a pepper crop grown in a closed hydroponic system as influenced by four cultivated varieties. i.e. ‘Orangery’ (O), ‘Bellisa‘(F), ‘Sondela’ (G) and ‘Sammy’ self-grafted (SS) or ‘Sammy’ grafted onto a commercial rootstock `RS10' (SG). Vertical bars indicate ± standard errors of means

The Ca concentration in the recirculating NS was appreciably reduced in the time between the start of anthesis and the start of harvesting (Figure 3.4C), because the uptake of Ca per L of water was maximized (Figure 3.4D), irrespective of the cultivar. The increased Ca uptake during this developmental stage resulted in higher leaf Ca concentrations at commencement of harvesting in comparison with the vegetative growth stage (Figure 3.4A). After commencement of harvesting, the Ca UC tended to decrease gradually (Figure 3.4D), and this resulted in increased Ca levels in the RNS, although the leaf Ca concentrations did not decrease. The fruit Ca concentrations increased during the late cultivation period, regardless of the root and shoot genotype (Figure 3.4B).

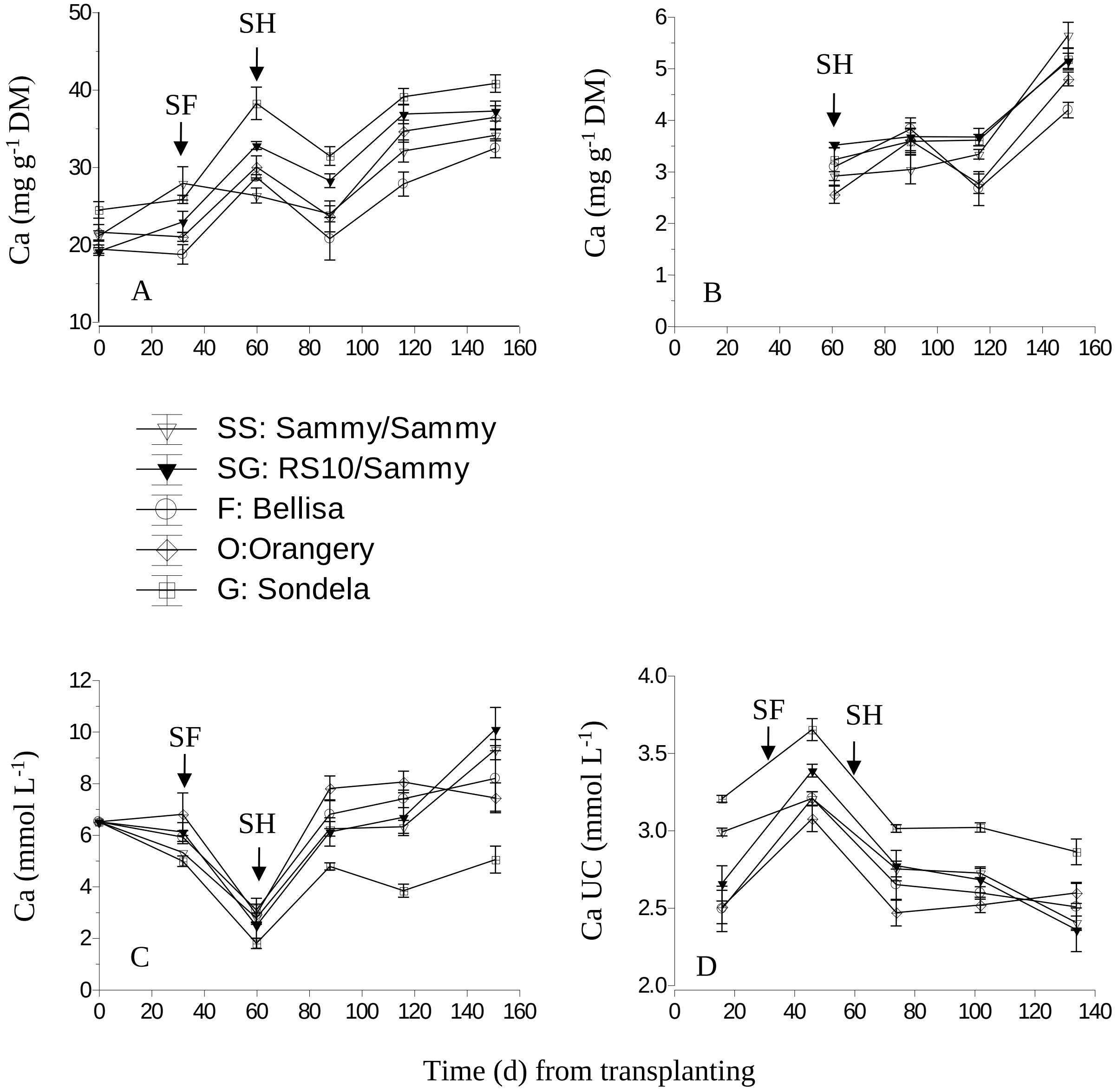

Figure 3.4. Impact of different pepper cultivars (‘Orangery’, ‘Bellisa‘, ‘Sondela’, ‘Sammy’) and grafting (‘Sammy’ self-grafted or grafted onto the commercial rootstock `RS10') on: i) concentrations of Ca in the dry mass (DM) of young leaves (A) and fruit (B), ii) concentrations of Ca in the recirculating nutrient solution (C), and iii) apparent Ca uptake concentrations (UC), i.e. mmol of Ca uptake per L of water uptake (D). Vertical bars indicate ± standard errors of means. SF: start of flowering; SH: start of harvesting.

The Mg concentration in the recirculating NS tended to decrease slightly up to the start of harvesting but thereafter it showed an increasing tendency which was very weak in ‘Sondela’ and stronger in ‘Sammy’, regardless of the root genotype (Figure 3.5C).

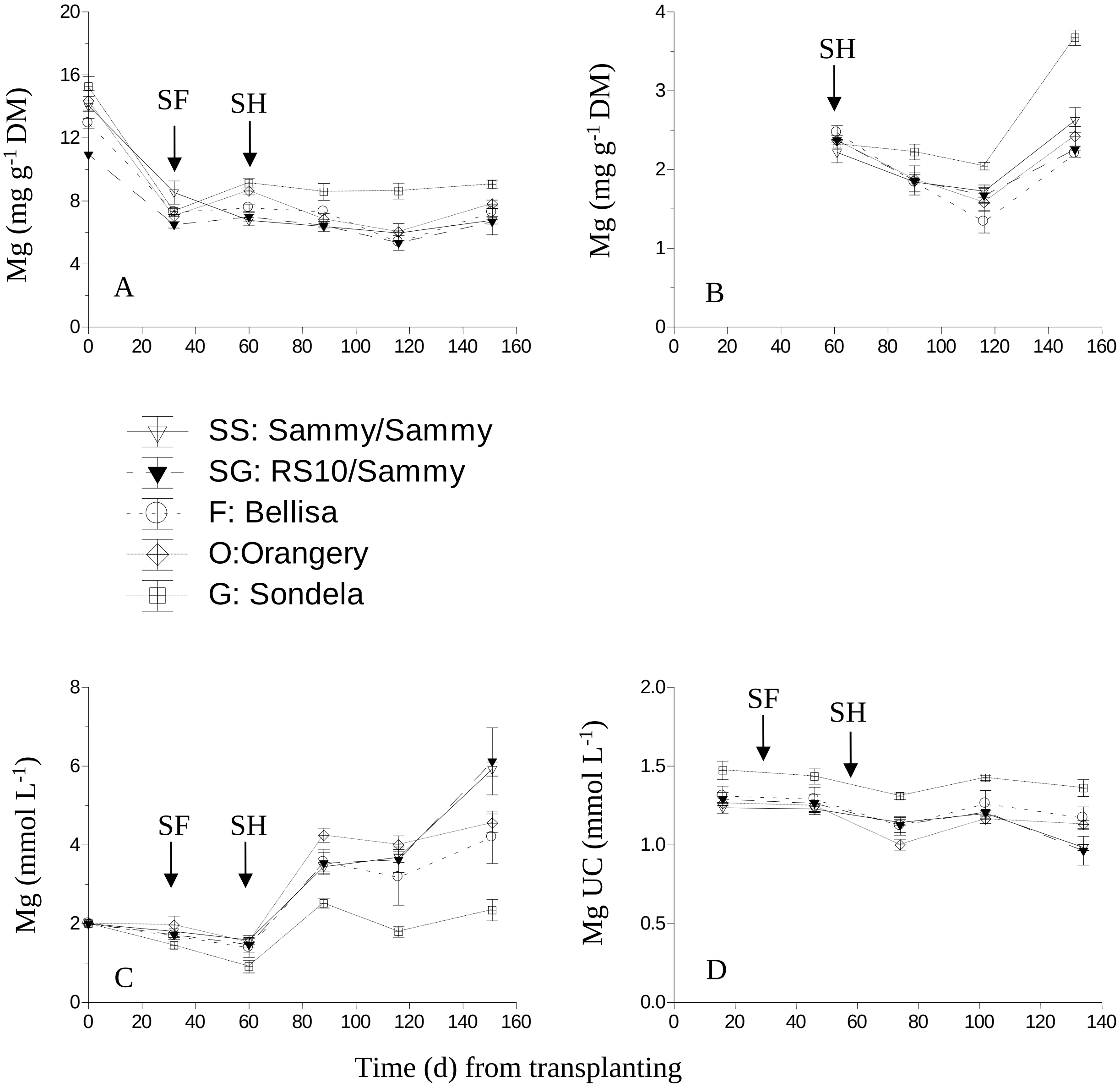

Figure 3.5. Impact of different pepper cultivars (‘Orangery’, ‘Bellisa‘, ‘Sondela’, ‘Sammy’) and grafting (‘Sammy’ self-grafted or grafted onto the commercial rootstock `RS10') on: i) concentrations of Mg in the dry mass (DM) of young leaves (A) and fruit (B), ii) concentrations of Mg in the recirculating nutrient solution (C), and iii) apparent Mg uptake concentrations (UC), i.e. mmol of Mg uptake per L of water uptake (D). Vertical bars indicate ± standard errors of means. SF: start of flowering; SH: start of harvesting.

On the other hand, the highest leaf Mg concentrations (Figure 3.5A) and Mg UC (Figure 3.5D) were measured in ‘Sondela’ throughout the cropping period. The leaf Mg concentrations were significantly higher at the fully vegetative stage (immediately after planting) than at later developmental stages, regardless of the root and shoot genotype. After commencement of flowering, both the leaf Mg concentrations and the Mg UC (Figure 3.5D) remained more or less constant during the whole cropping period. The fruit Mg concentrations tended to decrease up to day 120 from treatment initiation but this tendency was reversed at the late growing period.

‘Sondela’ exhibited consistently higher fruit Mg concentrations after commencement of harvesting in comparison with the other cultivars, while grafting had no impact on the fruit Mg level (Figure 3.5B).

The K concentration in the recirculating NS tended to increase slightly after the start of flowering and strongly after commencement of harvesting and up to 85 days after planting (Figure 3.6C). This increase in the recirculating NS was combined with a decrease in the leaf K concentration (Figure 3.6A) and the K UC (Figure 3.6D). Nevertheless, thereafter, both the leaf K concentration and the K UC increased again to similar levels with those measured in the interval between commencement of flowering and harvesting. Belissa exhibited consistently higher UC than the other cultivars throughout the cropping period and this resulted in lower K concentrations in the recirculating NS and higher leaf K concentrations after the start of flower. In contrast, ‘Orangery’ exhibited lower K UC after the start of harvesting but the differences were not always significant. The fruit K concentrations tended to increase slightly after commencement of harvesting and up to 85 days after treatment initiation, with the exception of ‘Sondela’ and ‘Belissa’ which exhibited lower and higher fruit K concentrations, respectively, than the other cultivars, although this was not consistent during the whole cropping period (Figure 3.6B).

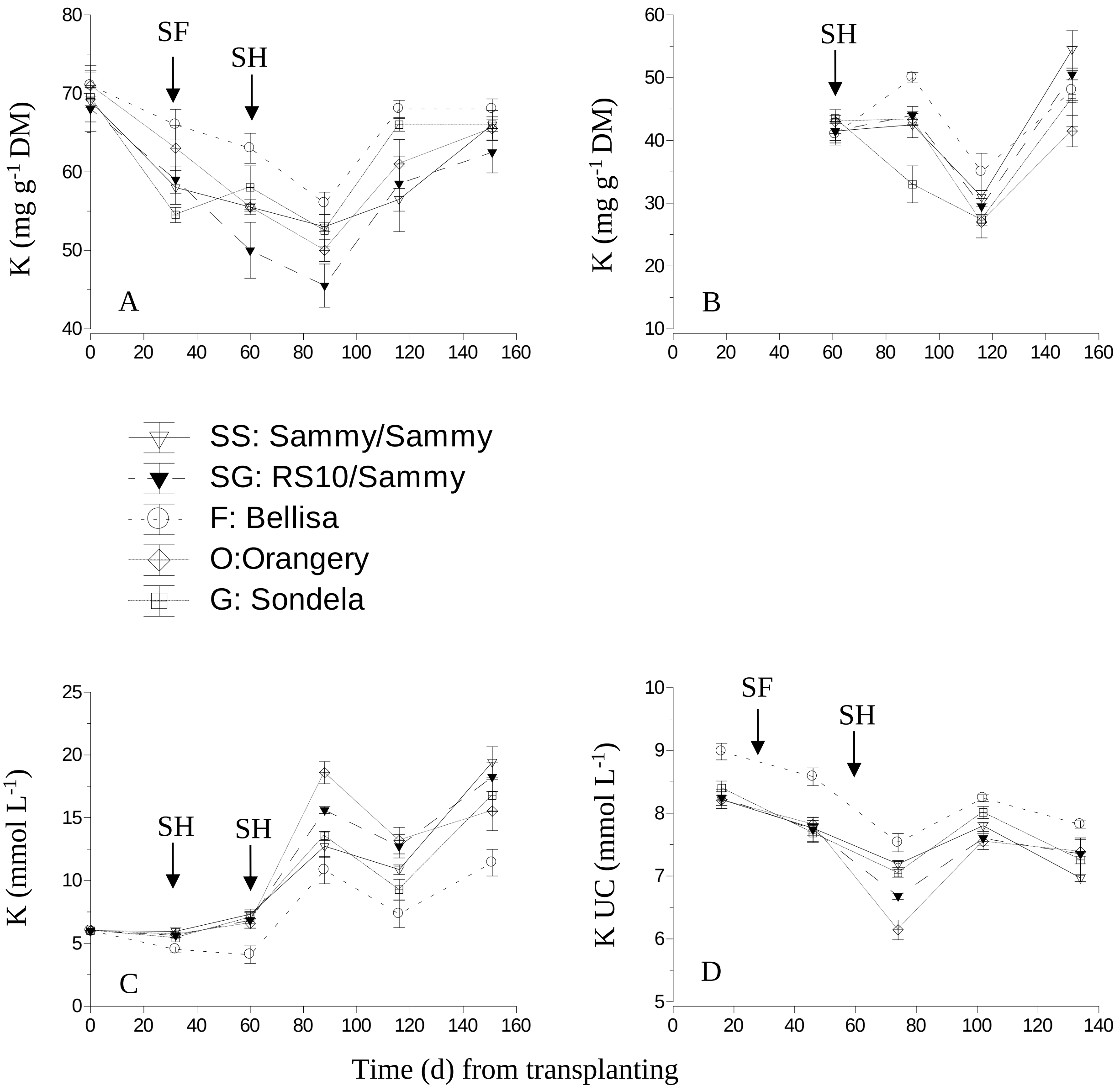

Figure 3.6. Impact of different pepper cultivars (‘Orangery’, ‘Bellisa‘, ‘Sondela’, ‘Sammy’) and grafting (‘Sammy’ self-grafted or grafted onto the commercial rootstock `RS10') on: i) concentrations of K in the dry mass (DM) of young leaves (A) and fruit (B), ii) concentrations of K in the recirculating nutrient solution (C), and iii) apparent K uptake concentrations (UC), i.e. mmol of K uptake per L of water uptake (D). Vertical bars indicate ± standard errors of means. SF: start of flowering; SH: start of harvesting.

The total nitrogen (NO_3-N and NH_4-N) concentrations in the recirculating NS solution (Figure 3.7C) and the leaves of pepper (Figure 3.7A) showed a slight decreasing tendency during the cropping period, while the total-N UC followed an increasing course up to the commencement of harvesting and shortly thereafter (Figure 3.7B). However, the total-N UC remained constant during the harvesting period. The self-grafted ‘Sammy’ plants tended to take up less N per L of water after the start of flowering and at the very early stage of harvesting but thereafter the N UC were not influenced by the pepper cultivar. The fruit N concentrations were not measured due to a technical failure which resulted in impairment of the samples.

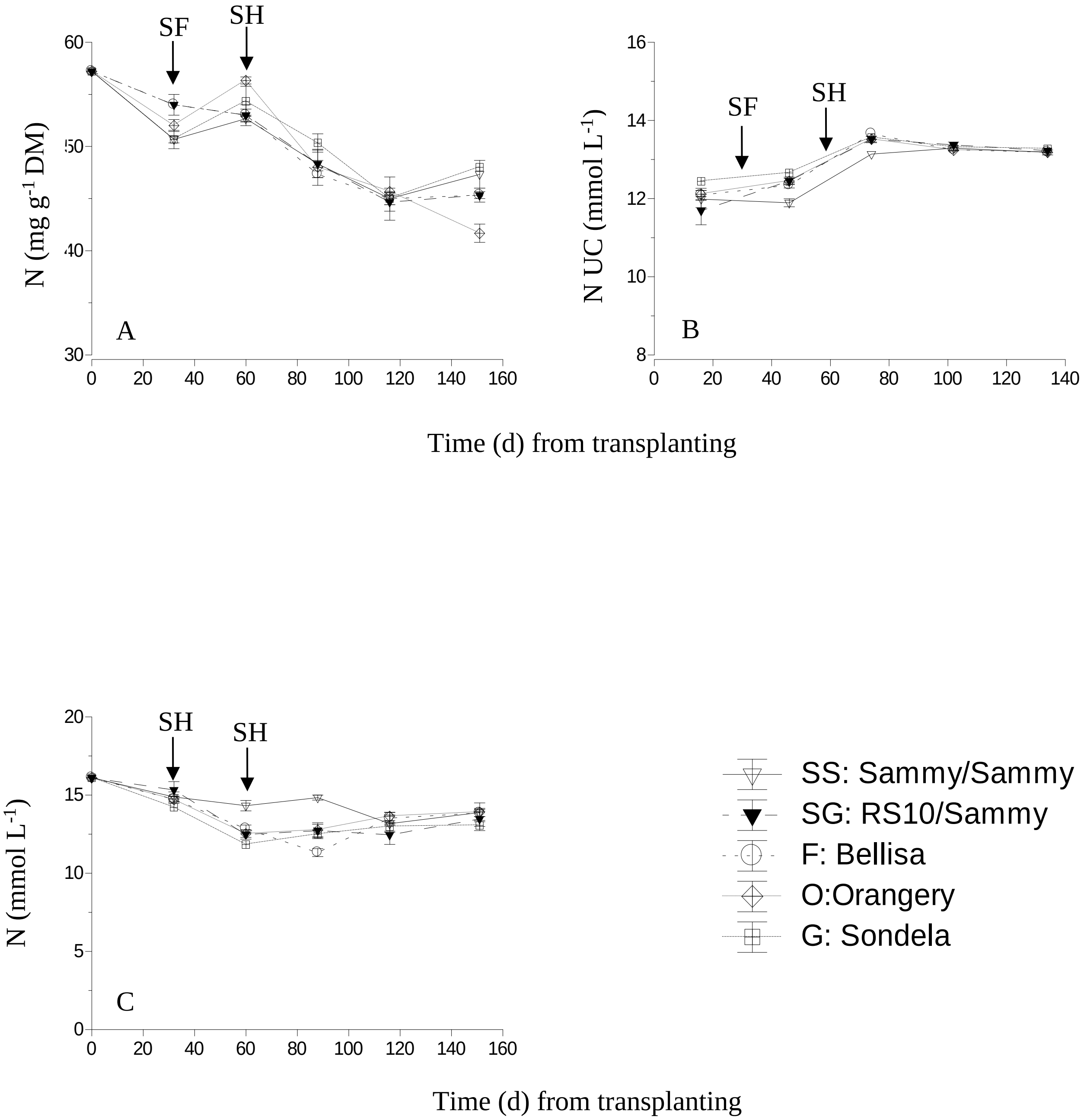

Figure 3.7. Impact of different pepper cultivars (‘Orangery’, ‘Bellisa‘, ‘Sondela’, ‘Sammy’) and grafting (‘Sammy’ self-grafted or grafted onto the commercial rootstock `RS10') on: i) concentrations of total N in the dry mass (DM) of young leaves (A), ii) apparent total N uptake concentrations (UC), i.e. mmol of total N uptake per L of water uptake (B), and iii) concentrations of total N in the recirculating nutrient solution (C). Vertical bars indicate ± standard errors of means. SF: start of flowering; SH: start of harvesting.

The P concentration in the recirculating NS showed an increasing tendency in the interval from planting up to the start of flowering in self-grafted ‘Sammy”, ‘Orangery’ and ‘Sondela’ (Figure

3.8C) which was accompanied by a commensurate decrease in the P UC at the same time interval (Figure 3.8D).

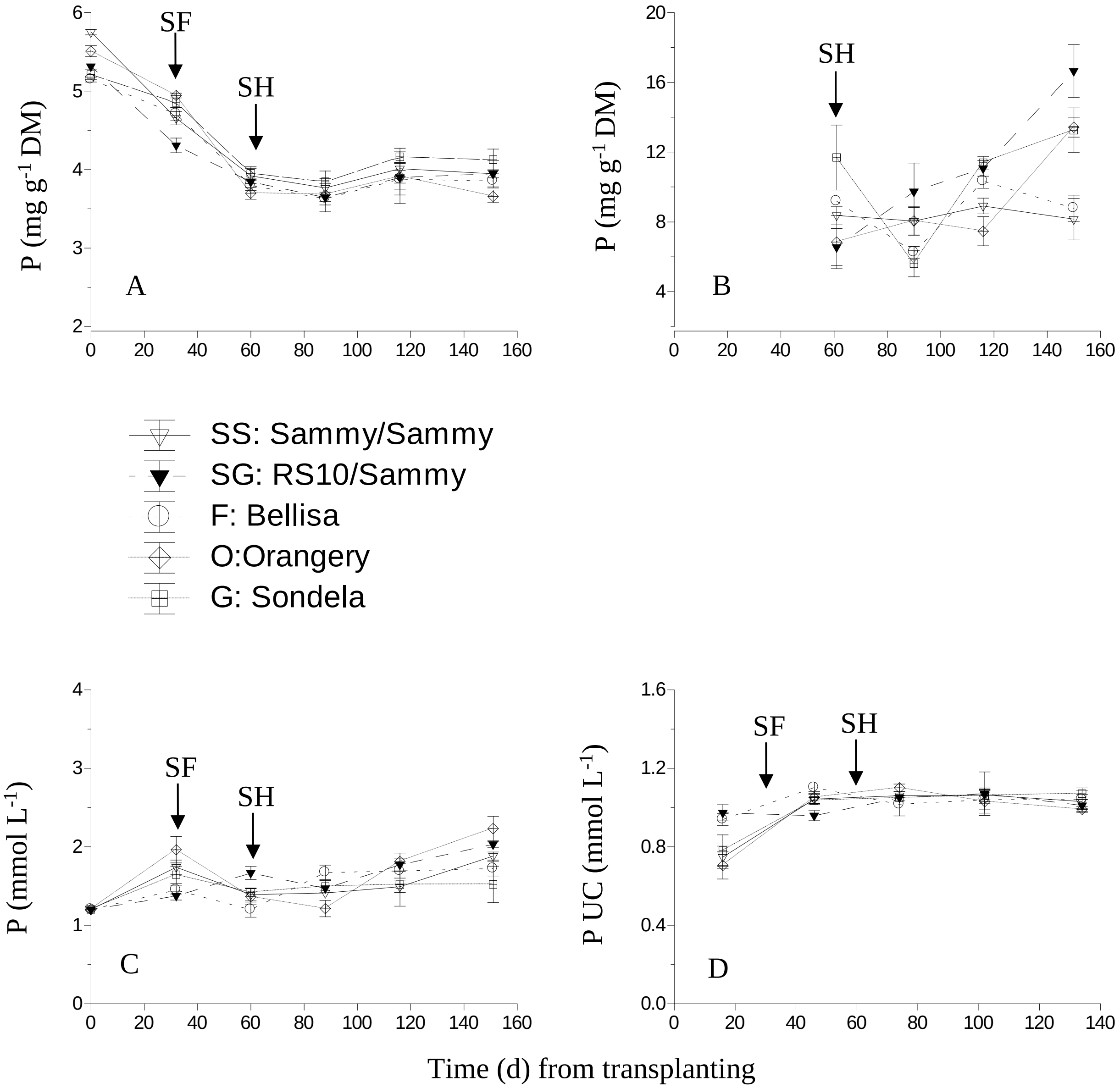

Figure 3.8. Impact of different pepper cultivars (‘Orangery’, ‘Bellisa‘, ‘Sondela’, ‘Sammy’) and grafting (‘Sammy’ self-grafted or grafted onto the commercial rootstock `RS10') on: i) concentrations of P in the dry mass (DM) of young leaves (A) and fruit (B), ii) concentrations of P in the recirculating nutrient solution (C), and iii) apparent P uptake concentrations (UC), i.e. mmol of P uptake per L of water uptake (D). Vertical bars indicate ± standard errors of means. SF: start of flowering; SH: start of harvesting.

In contrast, the P UC and the P levels in the recirculating NS of ‘Sammy’ grafted onto RS10 and ‘Belisa’ did not change by the time of flowering commencement in comparison to the initial vegetative growth stage. After the start of flowering, both the P concentration in the recirculating NS and the P UC remained more or less constant up to crop termination. Nevertheless, the leaf P concentration showed a consistent tendency to decrease after planting in all cultivars, which lasted up to the start of flowering (Figure 3.8A). The fruit P concentrations did not differ significantly between the different experimental treatments while it exhibited no consistent tendency during the cropping period (Figure 3.8B).

For micronutrients, only the UC are shown (Figure 3.9). The UC of Mn and Zh were highest during the vegetative growth stage and decreased slightly but constantly after commencement of harvesting. No consistent differences in UC between the different cultivars were found, although 'Orangery' showed a lower UC than the other cultivars during the vegetative stage (Figure 3.9A) and 'Sondela' showed a higher UC than the other cultivars (Figure 3.9B) in the interval between the start of flowering and the start of harvesting. The Fe UC also decreased constantly during the cropping period from 19.5 to 16 μmol L^{-1} on average, without any significant differences between the tested cultivars (Figure 3.9C). The B UC decreased from 27.6 μmol L^{-1} on average during the vegetative growth stage to about 25 μmol L^{-1} after the start of harvesting and remained roughly constant to these levels during the reproductive stage (Figure 3.9D). So significant differences between treatments could be found during the vegetative stage of growth, while during the late reproductive stage (after the first three months from planting) the B UC of 'Sondela" was significantly higher than those of the other tested cultivars. Grafting of 'Sammy' onto the rootstock RS10 had no significant impact on the UC of micronutrients.

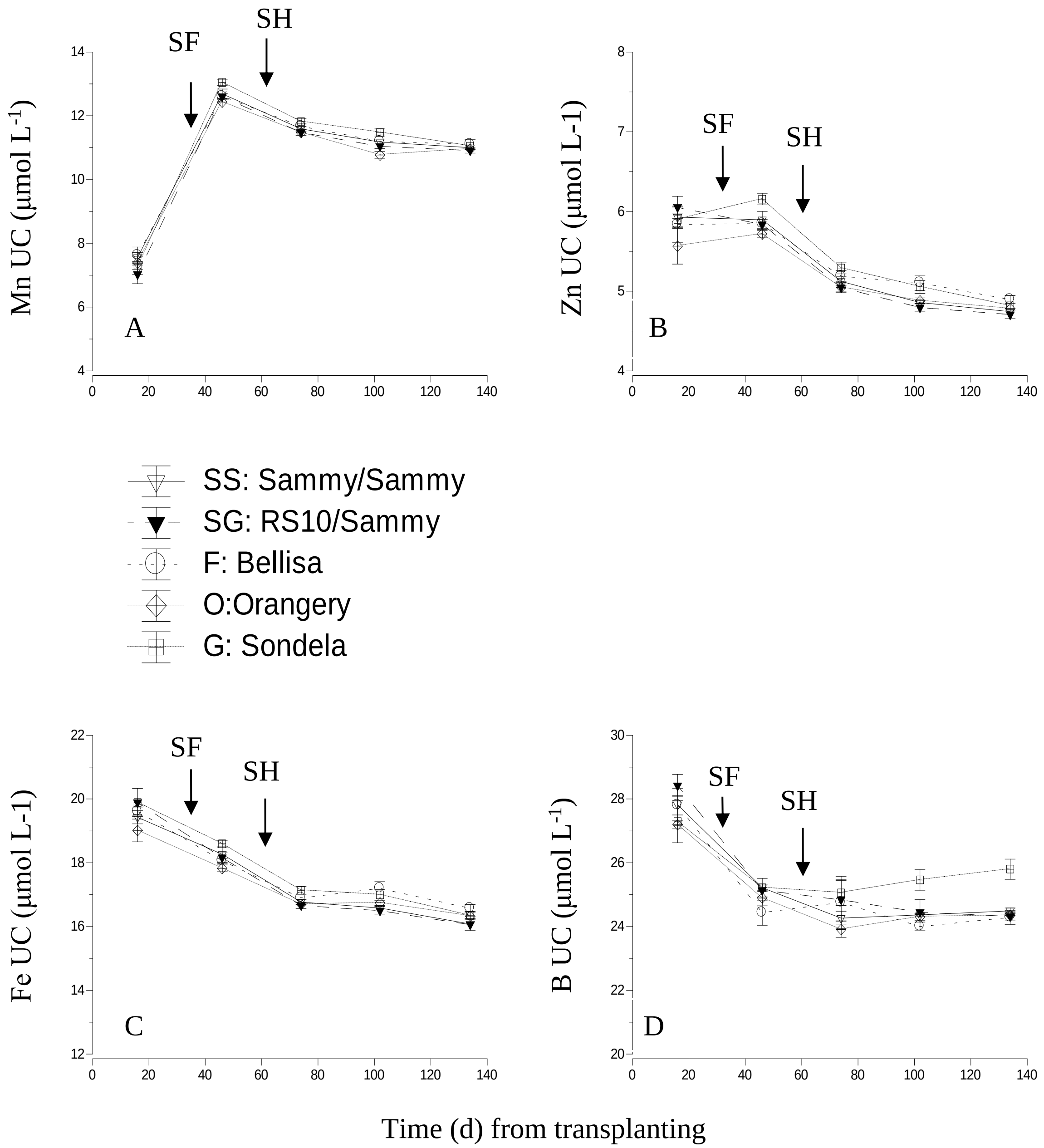

Figure 3.9. Estimated apparent uptake concentration of micro-nutrient (UC), (i.e. mmol of micro- nutrient uptake per L of water uptake), as influenced by four cultivated varieties. i.e. ‘Orangery’ (O), ‘Bellisa‘(F), ‘Sondela’ (G) and ‘Sammy’ self-grafted (SS) or ‘Sammy’ grafted onto a commercial rootstock `RS10' (SG). UC was calculated on the basis of (A) manganese (Mn), (B) zinc (Zn), (C) iron (Fe) and (D) boron (B) and water removal from the recirculating nutrient solution. Vertical bars indicate ± standard errors of means. SF: start of flowering; SH: start of harvesting.

3.5 Discussion

The present study showed that the developmental stage of the pepper plants has a strong impact on the nutrient to water uptake ratios (uptake concentrations: UC). For most nutrients, i.e. K, Ca, Mg and all micronutrients studied, the UC tended to decrease with time and only the UC of total-N and

P increased slightly after the initial vegetative stage. By definition, the UC (nutrient to water uptake ratios) depend on two variables, i.e. the rates of nutrient uptake and the rates of water uptake, which are independent to each other, since the plant metabolism is physiologically not related to transpiration (Taiz and Zaiger 2002). Thus, any difference in the UC at different plant developmental stages or between different cultivars may be due to commensurate differences either in the nutrient uptake rates, or in the water uptake rates, or in both. As the plants are growing up, the leaf area increases and this results in increased rates of whole plant transpiration (Taiz and Zaiger 2002). On the other hand, an increased leaf area results in increased rates of whole plant photosynthesis (Koyama and Kikuzawa 2009), thereby raising the nutrient demand and concomitantly the nutrient uptake rates. As a result, although the nutrient uptake and the water uptake are independent processes, the UC do not change dramatically during the cropping period, as has been shown in several studies (Savvas & Lenz 1995, Sonneveld & Voogt 2009). However, morphological changes at different plant developmental stages may differently affect the nutrient uptake rates than the water uptake rates. Thus, at the early plant developmental stages, all or most leaves are young, while at later developmental stages, when the plants enter the reproductive phase, a considerable part of the foliage consist of older leaves (Paltridge and Denholm 1974). The rates of transpiration are much less affected by the age of the leaves than the rates of net photosynthesis. Consequently, as the age of the plants increases, the water uptake rates increase more than the nutrient uptake rates and thus the uptake concentrations decrease, although in absolute terms the nutrient demand increases. The tendency of the UC for most nutrients (except of N and P) to decrease with time in the present study seems to result mainly from a stronger increase in water uptake rates with time than in the nutrient uptake rates.

The exceptions formed by the UC of N and P, which increased slightly as the plants entered the reproductive phase, may indicate a stronger increase in the N and P demand during the reproductive stage than the increase in the whole plant transpiration. An increase in the P demand by pepper as the plants pass from the vegetative to the reproductive stage can be ascribed to the appreciably higher P concentrations in the fruit than in the leaves of pepper. Indeed, as reported by Silber *et al.* (2005), the P concentrations in the leaves of pepper ranged from 2.1 to 3.7 mg g^{-1}, depending on irrigation frequency and P application rate, while the corresponding values in the fruit ranged from 6.2 to 7.5 mg g^{-1}. Similar differences in the P concentration between leaves and fruit were found also in the present study (Figure 3.8). However, the nitrogen concentrations tend to be higher in the leaf than in the fruit (e.g. 48.6 vs. 37.7 mg g^{-1}, respectively, as reported by Bar-Tal *et al.* (2001).

Hence, the slight increase in the N UC as the plants entered the reproductive stage in the present study cannot be ascribed to increased fruit N concentrations compared to those in leaves. An alternative explanation is a possible increase in the rates of denitrification as the crop was aging, which can be ascribed to the gradual increase in the air temperature, given that the crop was established on 16 January and the commencement of the reproductive stage coincided with the advent of the spring season. Nitrogen losses through denitrification in hydroponic nutrient solutions may be considerable, reaching levels up to 20% of the total supply, as has been shown by Daum and Schenk (1998). According to the method applied in the present study to estimate UC, denitrification losses are taken as apparent N uptake and, therefore, they tend to increase the estimated UC.

Since the UC represent uptake ratios between a nutrient and water, significant differences in the UC of any nutrient between cultivars originate from differences either in whole plant transpiration owing to leaf area differences, or in the nutrient demand, or in a combination of them. Furthermore, any differences in the nutrient demand between cultivars are mainly due to commensurate differences either in tissue nutrient concentrations or in the fruit biomass. The significantly higher Ca and Mg UC by Sondela and K UC by Bellisa in comparison with those recorded in all other genotypes, which were consistent during the whole cropping period, are partly ascribed to lower whole plant transpiration (Figure 3.1). ‘Sondela’ and ‘Bellisa’ are cultivars grown for the production of red pepper fruits, which need longer time to ripen than those harvested green, as is the case with ‘Sammy’. It is well known that the delay in fruit harvesting, which increases the fruit load, retards the vegetative growth (Engels *et al.* 2012), thereby reducing the leaf area per plan and concomitantly the whole plant transpiration. Nevertheless, the selective increase of only the UC of Ca and Mg in Sondella and K in Bellisa suggests that not only the whole plant transpiration but also the nutrient requirements are different between different cultivars. Indeed, while the whole plant transpiration was lower in both ‘Sondela’ and ‘Bellisa’ in comparison to the other genotypes, the leaf Ca and Mg concentrations were higher only in ‘Sondela’ during the reproductive stage of development. Hence, the factor that ultimately imposed higher Ca and Mg UC in ‘Sondela’ than in the other genotypes was the higher concentration of Ca in the leaves, and Mg in both leaves and fruit of ‘Sondela’ (Figures 3.4B and 3.5B). The reduced Ca and Mg concentrations in the nutrient solution that was recirculating in circuits accommodating ‘Sondela’ are in agreement with the higher Ca and Mg UC found for this cultivar. The same applies also for K in ‘Bellisa’. The significantly higher K UC in ‘Belisa’ were also due to a combination of higher whole plant transpiration and higher leaf K concentrations as indicated by Figures 3.1 and 3.6A.

The UC of total-N and P do not seem to be influenced by the genotype of the cultivar. Finally, the UC of the micronutrients Fe, Mn, Zn, and B did not show a consistent influence of the genotype, although ‘Sondela’ exhibited significantly higher Zn and B UC than the other cultivars at certain developmental stages. Sondela exhibited higher boron UC only during the reproductive developmental stage, which points to an effect of the fruit load on boron uptake rather than a transpiration-related effect on B UC.

An appreciable body of related investigations has indicated that grafting may decrease the uptake of some nutrients while increasing the uptake efficiency for some other nutrients depending mainly on the rootstock genotype (Ruiz et al. 1997, Rouphael et al. 2008, Savvas et al. 2009, Colla et al. 2010). Therefore, grafting has been suggested as a means to limit nutrient and heavy metal toxicity, or to increase fertilizer use efficiency and prevent nutrient deficiencies in marginally fertile soils (Savvas et al. 2013, Rouphael et al. 2016). Many rootstocks used to graft vegetables are wild genotypes of the same species as the scion, relatives, or hybrids of them, which are characterized by more vigorous root systems than those of highly productive cultivars (Huang et al. 2010, Pico et al. 2017). However, the pepper rootstock RS10, which was used to graft ‘Sammy’ in the current research, belongs to the same species with the scion (*Capsicum annuum*) and not to any wild relative species of pepper characterized by a vigorous root system. This is presumably the reason for the lack of any differences in leaf and fruit nutrient levels as well as in UC between self-grafted ‘Sammy’ plants and ‘Sammy’ plants grafted onto the rootstock RS10.

Comparing the UC found in the present study with those found by Voogt and Sonneveld (1997) in North European hydroponic greenhouses reveals that they are influenced by the contrasting climatic conditions. Indeed, the UC found by Voogt and Sonneveld (1997) in a pepper crop grown in a closed rockwool system in the Netherlands, averaged for the whole cropping period, were 2.2 mmol L^{-1} for Ca, 0.78 mmol L^{-1} for Mg, 4.6 mmol L^{-1} for K, 10.30 mmol L^{-1} for N, and 0.81 mmol L^{-1} for P. The corresponding UC found in the present study ranged between 2.4 to 3.7 mmol L^{-1} for Ca, 1.0 to 1.5 mmol L^{-1} for Mg, 6.1 to 9 mmol L^{-1} for K, 11.7 to 13.7 mmol L^{-1} for N, and 0.7 to 1.1 mmol L^{-1} for P.

A comparison between the values found in the two different studies reveals that the UC of macronutrients found in the present study are clearly higher than those reported by Voogt and Sonneveld (1997). In a similar study (Savvas *et al.* 2017), tomato plants grown under

Mediterranean climatic conditions had an increased need for N, P, K, Ca, Zn, and Cu and a decreased need for Fe, Mn, and B in comparison with the UC reported under North European climatic conditions (Voogt and Sonneveld 2009). The differences in UC between greenhouses located in these two contrasting environments may originate from differences either in the mean light interception, as reported by Adams (1993), or in the method applied to determine the UC, as reported by Tzerakis *et al.* (2013), Neocleous and Savvas (2015) and Savvas *et al.* (2017). The method applied in the present study to determine the UC takes into consideration possible precipitation losses. Therefore, the obtained values should be correctly termed "apparent uptake concentrations (Adams 2002). However, for a sufficient supply of nutrients to plants grown in closed hydroponic systems, it is essential to provide the amount of nutrients corresponding not only to net uptake but also to losses through precipitation or immobilization that may occur during the flow of the NS along the system. Therefore, the apparent nutrient UC determined in the present study constitute a sound basis to establish suitable NS compositions for pepper crops cultivated in closed hydroponic systems under Mediterranean climatic conditions.

3.6 Conclusion

The results of the present study indicated that different pepper cultivars may take up nutrients and water at different ratios under the same nutritional, irrigation and climatic conditions, as indicated by the observed differences in the UC (uptake concentrations). More specifically, 'Sondela' exhibited the highest Mg and Ca UC throughout the cropping period and B UC during the reproductive stage in comparison with all other tested cultivars. Furthermore, 'Belissa' exhibited significantly higher K UC throughout the cropping period in comparison with all other tested cultivars.

The tissue nutrient concentrations and the UC were similar in 'Sammy' self-grafted and 'Sammy' grafted onto the commercial rootstock `RS10' (*Capsicum annuum*), which indicates that this Capsicum annum rootstock does not modify the uptake of nutrients and water by the scion. The developmental stage of the pepper plants had a strong impact on the UC of most nutrients due to changes in the mean physiological age of the leaves, the onset of fruit production and seasonal differences in climatic conditions.

Based on the UC estimated in the present study, the nutrient solutions supplied to closed hydroponic crops of pepper should contain Ca, Mg and B at higher concentrations than standard recommendations when the cultivated variety is ‘Sondela’ and K at higher concentrations when the cultivated variety is ‘Bellisa’.

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CHAPTER 4. RESPONSES OF SWEET PEPPER (*Capsicum annum* L.) CULTIVATED IN A CLOSED HYDROPONIC SYSTEM TO VARIABLE CALCIUM CONCENTRATIONS IN THE SUPPLIED NUTRIENT SOLUTION

4.1 Abstract

In the Mediterranean regions, the available irrigation water frequently contains sodium chloride but also calcium bicarbonate at excessively high concentrations. The use of water containing calcium bicarbonate ($Ca(HCO_3)_2$) at excessively high concentrations in closed hydroponic crops can cause calcium ion (Ca) accumulation in the recycled nutrient solution (NS) and concomitantly negatively affect yield and product quality. The aim of the study was to determine maximum Ca concentrations that do not harm the crop and to simulate the pattern of Ca accumulation when the Ca concentration in the irrigation water, and concomitantly in the replenishment nutrient solution (RNS), is excessive. In the current study, irrigation water containing 1.5, 3.0, 4.5 and 6.0 mM Ca was used to prepare the RNS supplied to pepper cultivated in a closed hydroponic system. The “uptake concentrations” (UCs) of macronutrients (i.e., N, S, K, Ca and Mg) under conditions of progressive Ca accumulation in the recirculating NS and thus in the root zone were determined. Plant biomass production, fruit yield and quality were also recorded. At 1.5 mM Ca, no Ca accumulation was observed in the recirculating NS. However, at 3.0, 4.5 and 6.0 mM in the irrigation water, the Ca concentration in the recirculating NS, and concomitantly in the root environment, increased to 17, 28 and 37 mM, corresponding to 6.4, 9.0 and 10.8 dS m^{-1}. The accumulation of Ca in the recirculating NS affected both tissue nutrient concentrations and nutrient to water uptake ratios (uptake concentrations) of Ca, S and Mg, but this was not the case for N and K. Growth, yield and plant water uptake were restricted at moderate (3.0 and 4.5 mM) and high (6.0 mM) external Ca levels, as pepper is susceptible not only to salinity but also to nutrient imbalances in the root environment. Our results showed that in soilless sweet pepper crops with zero discharge of fertigation effluents, the Ca concentration in the irrigation water and the RNS should be lower than 3.0 mM to avoid yield restrictions due to salinity.

4.2 Introduction

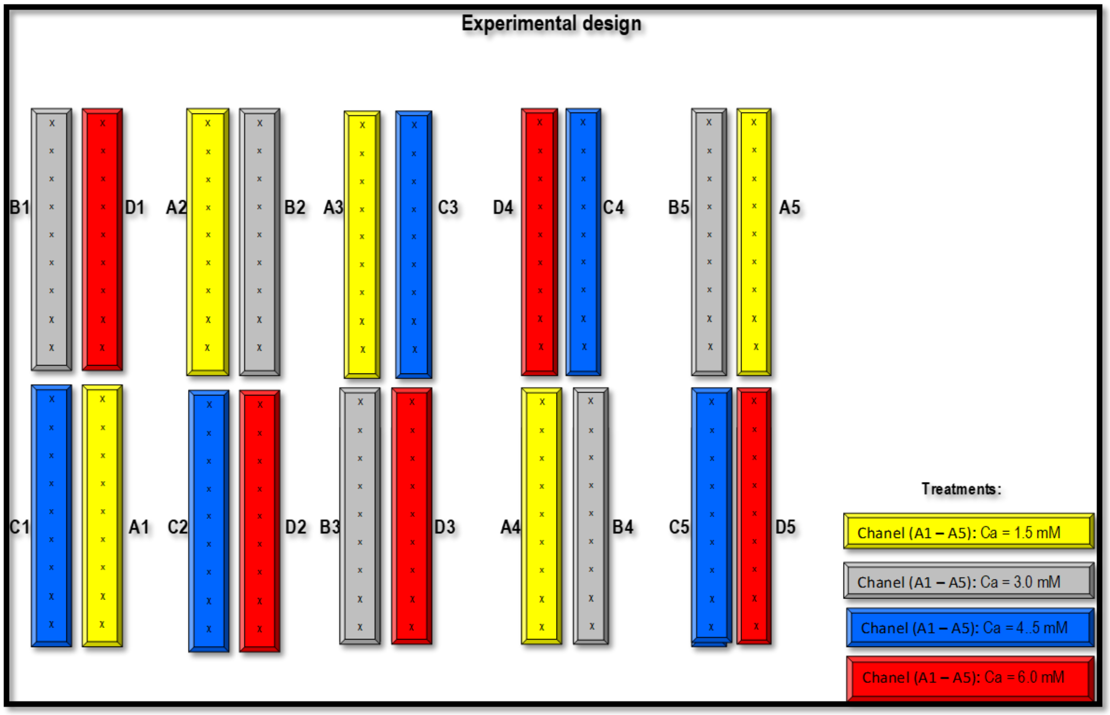
In open soilless crops, the fraction of nutrient solution (NS) that drains out of the root zone after each irrigation event to leach salts from the rhizosphere and ensure sufficient water supply to all plants is wasted (Massa *et al.* 2020). To save precious water and fertilizers resources and avoid adverse environmental impacts due to pollution of groundwater with nitrates, governmental authorities have put increasing pressure to growers through legislation to switch over to closed soilless cultivation systems (Sonneveld et al. 2002, Ropokis et al. 2018). A common problem encountered in closed hydroponic systems, especially when the source water is of poor quality, is the accumulation of ions, including non-nutrients which may be toxic (e.g. sodium), but also nutrients contained at excessive concentrations compared to plant requirements, particularly calcium ion (Ca), magnesium ion (Mg) and sulphate ion (SO_4). More specifically, this is the case when the ion to water inlet ratios (i.e. concentrations in the irrigation water) are higher than the corresponding ion to water uptake ratios (Massa *et al.* 2020). The accumulation of nutrient ions to excessive levels results in an unbalanced ratio between nutrients and a higher electrical conductivity (EC) in the NS surrounding the roots, and concomitantly in reduced yield (Ropokis *et al*. 2018).

The ion to water uptake ratios, which are termed 'uptake concentrations' (UC) (Adams 2002, Sonneveld 2002, Sonneveld & Voogt 2009), exhibits an appreciable stability over time under similar climatic conditions for a specific plant species and developmental stage, if the ionic concentrations in the root zone are relatively stable over time (Sonneveld & Voogt 2009, Tzerakis et al. 2013, Savvas & Gruda 2018). However, the concentration of a nutrient (e.g. Ca) in the available irrigation water may be higher than the mean nutrient to water uptake ratio (Neocleous and Savvas 2018). In these cases, this nutrient and the counterbalancing nutrient ion(s), gradually accumulate in the root zone and concomitantly the UCs of these nutrients are also altered (Neocleous and Savvas 2018). Furthermore, the accumulation of this nutrient in the root zone may also affect the UCs of other nutrients due to ionic competition in uptake, thereby negatively affecting crop nutrition (Varlagas *et al*. 2010). To assess this hypothesis, UCs of Ca, Mg, potassium ion (K), nitrate (NO_3), ammonium cation (NH_4) and SO_4 were determined in a closed hydroponic crop of pepper, under conditions of progressive Ca accumulation in the root zone imposed by excessively high Ca concentrations in the available irrigation water. Finally, the impact of altered UCs and nutrient accumulation in the root zone of pepper on plant biomass and fruit yield was also studied. The overall objective of the current study was to provide specific knowledge needed to control efficiently Ca nutrition in pepper crops cultivated in closed hydroponic systems, especially when the Ca concentration in the irrigation water exceeds the Ca to water uptake ratio.

4.3 Materials and methods

4.3.1 Plant material and growth conditions

The experiment was conducted in a heated glasshouse at the Agricultural University of Athens (37°58'57.8"N, 23°42'14.3"E). Four experimental treatments were applied in a pepper crop, corresponding to four variable concentrations of Ca in the NS supplied to a pepper crop, particularly 1.5, 3.0, 4.5 and 6.0 mM Ca. For each treatment, five experimental closed hydroponic circuits were randomly distributed in the glasshouse and thus 20 experimental circuits were used as shown in Picture 5.



Picture 5. Pepper plants into channels of the glasshouse pruned according to the 'V' system.

This system was described in detail in a previous paper (Ropokis *et al.* 2018). The pepper (*Capsicum annuum* L. cv. 'Sammy') plants were grown in recirculating NS using a nutrient film technique (NFT). Each circuit comprised one channel, 3.0 m in length, 0.015 m in width, and 0.03 m in height placed at a slope of 1%, an individual supply tank at the lower end of the channel, a pump continually supplying NS via an irrigation pipe to the upper end of the channel, and an individual replenishment tank. The four different Ca concentrations were applied in the NS contained in the replenishment tank, which will be henceforth termed replenishment nutrient solution (RNS). Nine seedlings were transferred into each circuit on 11 September, at the stage of five to six true leaves, corresponding to a plant density of 2.5 plants per m^2. The pepper plants were pruned and supported according to the 'V' system, which resulted in a two-stem plant obtained by removing one of the two shoots developed at each node. The distance between individual plants in each channel of the circuit was 0.35 m. The crop was terminated on 25 February. In each experimental unit, the NS was automatically applied from the supply tank at a rate of 0.4 m^3 h^{-1}. The total amount of NS per plant in each experimental unit (including both the solution contained in the supply tank and that in recirculation) amounted to 3 L per plant. The NS consumed by the plants was automatically replaced by RNS coming from the replenishment tank, using a float valve. The RNS consumed by the plants was measured daily by recording the change in volume of the replenishment tank. All channels were covered with black–white polyethylene sheets to avoid water evaporation as shown in Picture 6.

Picture 6. Pepper plants into channels of the glasshouse pruned according to the 'V' system.

The mean monthly air temperatures in the greenhouse during the experiment are shown in Table 4.1. No drainage water was discharged and losses due to technical failures were negligible.

Table 4.1. Mean monthly temperatures in the greenhouse during the whole experiment.

Month	T (°C)
Sep	27.9
Oct	24.6
Nov	21.1
Dec	19.2
Jan	20.7
Feb	21

Before transplanting, all supply tanks were filled with a starter NS, while each replenishment tank was filled with one out of the four RNSs, corresponding to the four experimental treatments. Immediately after transplanting, the four different RNSs were supplied to the plants through the float valve in all circuits, to compensate for NS uptake by the plants.

4.3.2 Composition and sampling of experimental nutrient solutions

The nutrient concentrations in the starter NS followed a modified formula suggested for pepper (de Kreij *et al.* 1999) and were in all treatments as follows: 5.5 mM K, 6.5 mM Ca, 2.0 mM Mg, 1.1 mM NH_4, 16.4 mM NO_3, 1.2 mM H_2PO_4, 5.8 mM SO_4, 20.0 μM Fe, 10.0 μM Mn, 6.0 μM Zn, 0.8 μM Cu, 50.0 μM B, and 0.5 μM Mo. The EC and pH in the starter NS were 2.6 dS m^{-1} and 5.6, respectively. Henceforth, the calcium concentrations in the RNS are referenced as low (1.5 mM), medium (3.0 and 4.5 mM) and high (6.0 mM). The different total cation concentrations imposed by different Ca concentrations in each treatment, were counterbalanced by equivalent variations in the NO_3 and SO_4 concentrations. The nutrient concentrations in the four different RNSs are shown in Table 4.2.

Table 4.2 Nutrient concentrations in the four replenishment nutrient solutions (RNSs) applied as different experimental treatments.

	Ca concentrations in the RNS			
Nutrient	Low	Medium		High
Ca (mmol L^{-1})	1.5	3.0	4.5	6.0
SO_4 (mmol L^{-1})	0.7	1.2	1.8	2.2
NO_3 (mmol L^{-1})	11.5	13.5	15.5	17.5
K (mmol L^{-1})	8.0	8.0	8.0	8.0
Mg (mmol L^{-1})	1.1	1.1	1.2	1.1
NH_4 (mmol L^{-1})	1.0	1.0	1.0	1.0
H_2PO_4 (mmol L^{-1})	1.1	1.1	1.1	1.1
Fe (μmol L^{-1})	15.0	15.0	15.0	15.0
Mn (μmol L^{-1})	10.0	10.0	10.0	10.0
Zn (μmol L^{-1})	4.0	4.0	4.0	4.0
Cu (μmol L^{-1})	0.7	0.7	0.7	0.7
B (μmol L^{-1})	25.0	25.0	25.0	25.0
Mo (μmol L^{-1})	0.5	0.5	0.5	0.5

The pH in the recirculating nutrient solution was daily adjusted to 5.6-5.7 by adding appropriate amounts of 1 mol L^{-1} nitric acid (HNO3) stock solution based on the actual pH level, which was measured using a portable pH-meter (pH 25, Crison). The EC was also recorded daily using a portable EC-meter (CM 25, Crison, CRISON INSTRUMENTS S.A., Barcelona, Spain).

Immediately after transplanting, samples of starter NS were collected before its placement into the tanks. After transplanting, samples of recirculating NS were selected at the lower end of each channel in all closed hydroponic units on a 3-weekly basis until 25 February.

4.3.3 Yield, water consumption and water use efficiency

Harvesting commenced on October 15 in all treatments and terminated on February 25. Commercially ripe fruits were harvested twice per week to calculate the total number of fruit per plant, the total fresh yield and the mean fruit weight in each treatment. Crop water use efficiency (WUE) was calculated as fruit yield (kg/plant) divided by the cumulative water consumption (L/plant).

4.3.4 Determination of nutrient concentrations

The concentrations of Ca and Mg in both NSs and aqueous extracts of plant tissues were measured using an atomic absorption spectrophotometer (Perkin Elmer 1100A, Perkin Elmer, Waltham, MA, USA), while that of K was determined by flame photometry (Sherwood Model 410, Cambridge, UK). The concentrations of NO_3, NH_4 and S were measured photometrically using a 96-position microplate spectrophotometer (Anthos Zenyth 200; Biochrom, USA). NO_3 and NH_4 were determined by applying the copperized cadmium reduction method (Griess-Ilosvay procedure) at 540 nm, and the indophenol blue method at 630 nm, respectively. The NH_4 concentration was added to that of NO_3 in each sample to obtain the total N concentration. Total N in plant tissues was determined following the Dumas combustion method using a C/N elemental analyzer (Unicube, Elementar Analysensysteme GmbH, Hanau, Germany). Nutrient to water uptake ratios (UCs) for Ca, Mg, total N, K and SO_4 were estimated as described by Ropokis *et al*. (2018).

4.3.5 Estimation of mean uptake concentrations

Nutrient to water uptake ratios (uptake concentrations) for Ca, Mg, total N, K and S were estimated based on the removal of nutrients from the nutrient solution. In particular, the mean uptake concentration of the *x* micronutrient (C_{xu} in μmol L^{-1}, where *x* = Ca, Mg, N, K and S) was determined for two time intervals using the following mass balance equation:

$$C_{xu} = \frac{V_r(C_{xbi} - C_{xei}) + V_{ui}C_{xa}}{V_{ui}} \qquad (1)$$

where V_r (L) denotes the total volume of the recirculating NS in each experimental unit (70 L in the present study); V_{ui} (L) denotes the total volume of NS that was taken up by the plants in each experimental unit during the i time interval (i = 1...8); C_{xbi} and C_{xei} (μmol L^{-1}) denote the concentrations of the x nutrient in the recirculating NS on the first and the last day of the i time interval (i = 1...8); C_{xa} (μmol L^{-1}) denotes the concentration of the x nutrient in the RNS used in each treatment.

4.3.6 Statistical analysis

The four different NS treatments were applied as a randomized complete block experimental design. The impact of the different treatments was evaluated by applying one-way ANOVA. Multiple comparisons of means were performed by applying the Duncan's Multiple Range Test and the significance of differences for the results presented in figures is shown in Supplementary Table 1. The STATISTICA software package, version 12.0 (StatSoft, Inc., Tulsa, OK, USA) was used to statistically evaluate the data.

4.4 Results

4.4.1 Changes in cumulative water consumption and EC

The cumulative amount of water consumed by pepper throughout the cropping period was reduced at high Ca concentrations in the supplied NS. Particularly, on the last sampling date, 15%, 19% and 28% less water was consumed by plants grown under 3.0, 4.5 and 6.0 mM Ca in the NS respectively, when compared to the control treatment (i.e. 1.5 mM) as shown in Fig. 4.1. These differences were in line with clear differences between the treatments in the vegetative plant biomass and the leaf area (data not show) at the end of the experiment (i.e 168 days from transplanting).

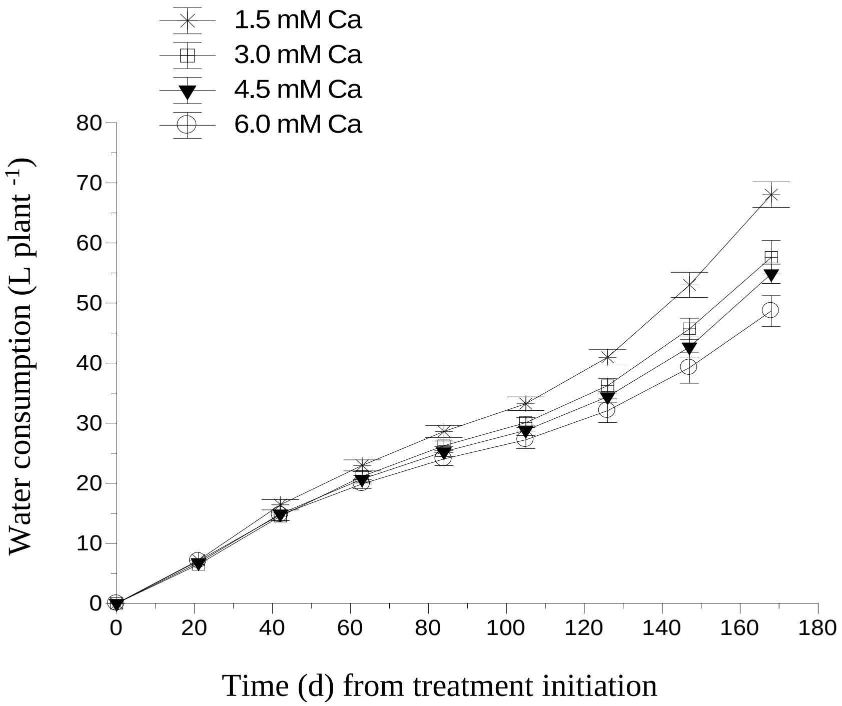


Figure 4.1. Evolution of cumulative water consumption (CWC) in a greenhouse crop of pepper (*Capsicum annum*, cv. ‘Sammy') grown in closed hydroponic systems as influenced by four different concentrations of Ca in the RNS. Vertical bars indicate ± standard errors of means.

The EC in the recirculating NS gradually increased with time in the medium and high-Ca treatments (3, 4.5 and 6 mM Ca in the RNS), while in the low Ca treatment (1.5 mM Ca in the RNS) the EC in the recirculating NS slightly decreased with time (Fig. 4.2.)

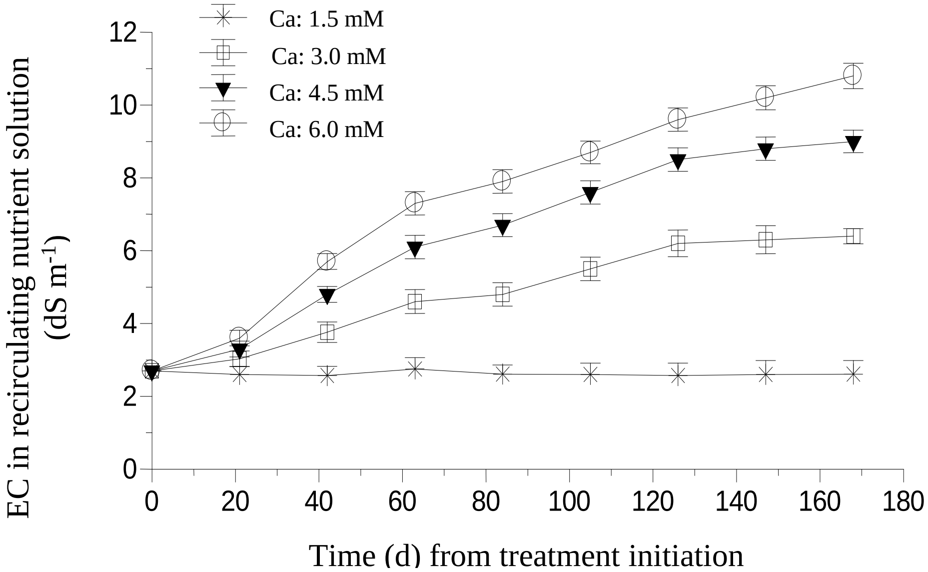


Figure 4.2. Electrical conductivity (EC) in recirculating nutrient solution in a greenhouse crop of pepper (*Capsicum annum*, cv. ‘Sammy') grown in closed hydroponic systems as influenced by four different concentrations of Ca in the RNS. Vertical bars indicate ± standard errors of means.

4.4.2 Fruit yield and water use efficiency

The total fruit yield decreased significantly as the Ca level in the RNS increased, due to commensurate restrictions in the number of fruit per plant, while the mean fruit weight was not influenced (Figure 4.3).

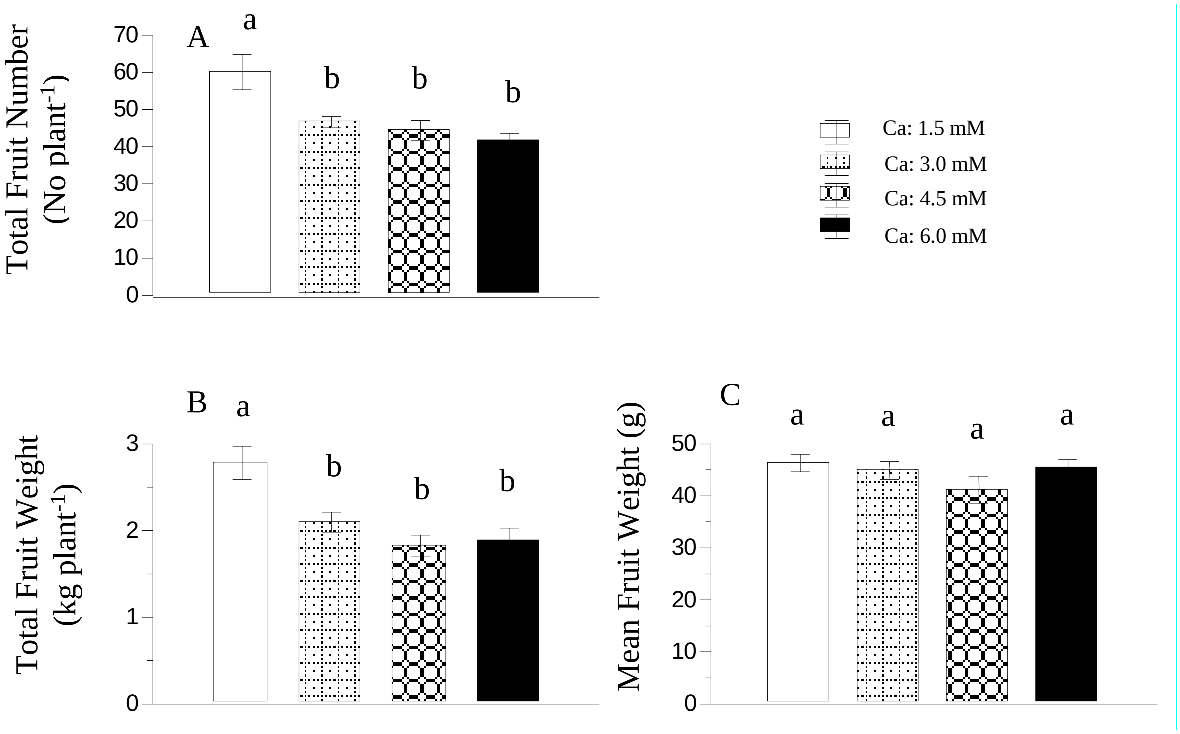


Figure 4.3. Influence of four different Ca concentrations in the RNS supplied to a greenhouse crop of pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems on: (a) total fruit number (TFN) per plant, (b) total fruit weigh (TFW) per pant and (c) average marketable fruit weigh (MFW) per plant. In each graph, different letters above bars indicate significant differences between means (n=5) according to the Duncan's Multiple Range Test. Vertical bars indicate ± standard errors of means

In particular, the total fruit number in the treatment with the lowest Ca concentration (i.e. 1.5 mM) in the RNS was 22% higher than in the 3.0 mM treatment, 26% higher than in the 4.5 mM treatment and 30% higher than in the 6.0 mM treatment. Accordingly, the total fruit weight in the treatment with the lowest Ca concentration in the RNS (i.e. 1.5 mM) was 24% higher than in 3.0 mM treatment, 34% higher than in 4.5 mM and 32% higher than in 6.0 mM treatment. However, as shown in Figure 4.4, the different Ca-supply treatments had no significant impact on WUE.

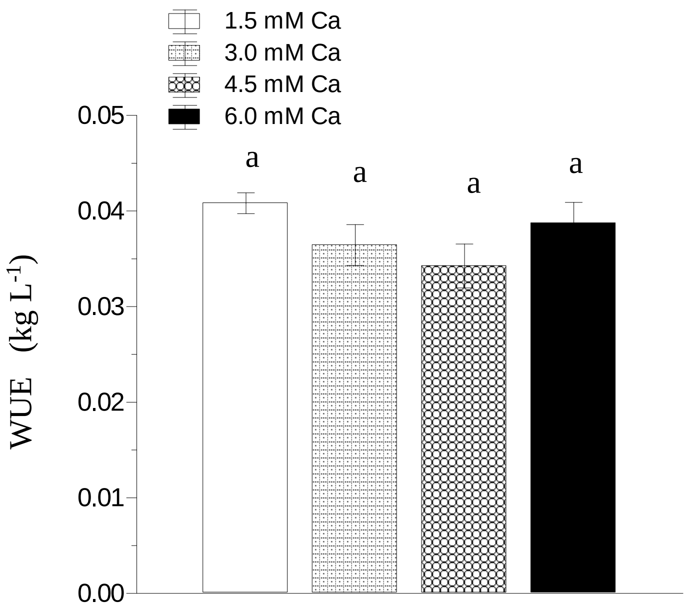


Figure 4.4. Estimated water use efficiency (WUE) as influenced by four different concentrations of Ca in the RNS supplied to the crop to compensate plant uptake in a greenhouse crop of pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems. Water use efficiency was calculated as fruit yield (kg/plant) divided by the cumulative water consumption (L/plant). Vertical bars indicate ± standard errors of means

The quadratic (second-order) polynomial model ($y=b_0+b_1x+b_2x^2$) was used as a model to describe the independent variable (total yield) as a relationship of the dependent variable (Ca in the RNS). According to this model, which is shown in Supplementary Figure 4a, significantly higher yield (2.78 kg plant^{-1}) was observed at 1.5 mM Ca in the RNS compared to the other three treatments with higher Ca concentrations. However, no significant differences could be found between the three high-Ca treatments.

4.4.3 Nutrient concentrations and uptake

The evolution of Ca accumulation with time in the recirculating NS is presented in Fig. 4.5a. Although, the same accumulation pattern (sigmoid curve) was observed for the cumulative water consumption, clear differences in Ca accumulation levels over the whole cultivation cycle were observed among treatments. In the medium and high Ca treatments, the Ca concentrations in the recirculating NS were increasing during the whole experimental period but a sharper increase was observed 22 days after treatment initiation (DAT). However, in the low Ca treatment, the Ca concentration in the recirculating NS slightly decreased at the start of anthesis, while at 95 DAT the Ca levels showed a tendency to stabilize to a minimal level. Finally, Ca ions accumulated in the drainage solution up to 2.5, 17.2, 27.7 and 37.2 mM, in the treatments with Ca concentrations 1.5, 3.0, 4.5 and 6.0 mM in NS, respectively.

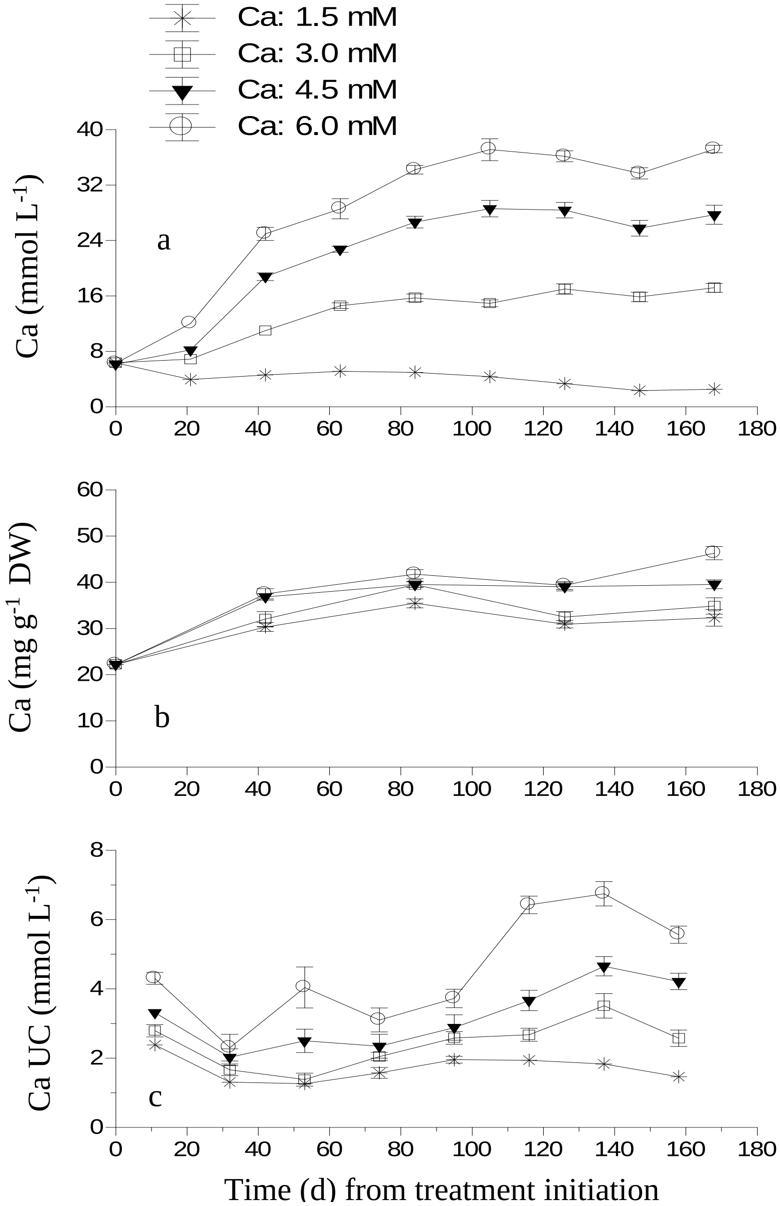


Figure 4.5. Concentrations of Ca: (a) in recirculating nutrient solution (NS), (b) in the dry biomass (DW) of leaves removed from pepper plants and (c) apparent uptake concentration (UC) of Ca (Ca uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum*, cv. ‘Sammy') grown in closed hydroponic systems. Vertical bars indicate ± standard errors of means.

In leaves, the Ca concentrations increased in all treatments by the increase of Ca concentration in the supplied NS (Fig. 4.5b). Based on the removal of nutrient from NS and crop water uptake, the effect of different external Ca concentrations on the mean Ca uptake concentrations (UCs; amount of nutrient absorbed per volume of water consumption) was influenced by NS treatments. Particularly, the UC of Ca increased with time when the Ca concentration in the NS was 4.5 or 6.0 mM, while it slightly decreased when the Ca concentration in the NS was 1.5 or 3.0 mM (Fig 4.5c). In all treatments, the Ca UCs were high during the vegetative stage and decreased as the plants passed to the reproductive stage

The NO_3, and concomitantly the total N concentration in the recirculating NS increased during the experimental period only when the Ca concentrations were 3.0, 4.5 and 6.0 mM in the RNS. However, the accumulation rate of total N slightly decreased when the Ca concentration in the RNS was 1.5 mM after the initiation of the experimental period, with a tendency to stabilize to a minimal level towards the end of the experiment (Fig. 4.6a). The concentrations of total N in the leaves were reduced with time, irrespective of the Ca supply treatments (Fig. 4.6b). The mean UCs of N, during the whole experimental period, ranged between 11.0 - 11.7 mmol L^{-1} [1.5 mM Ca], 12.2 -13.4 mmol L^{-1} [3 mM Ca], 13.2 - 14.2 mmol 291 L^{-1} [4.5 mM Ca], and 14.2 - 15.7 mmol L^{-1} [6 mM Ca] (Fig. 4.6c).

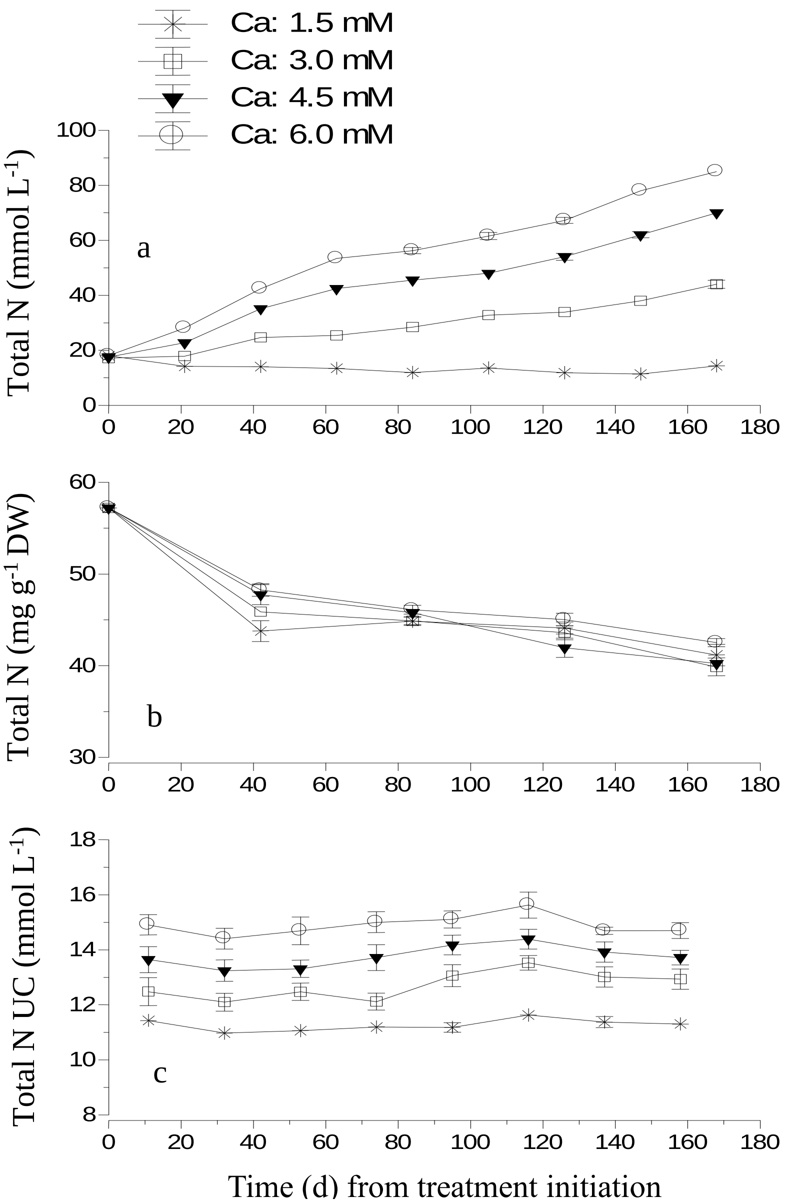


Figure 4.6. Concentrations of total N: (a) in recirculating nutrient solution (NS), (b) in the dry biomass (DW) of leaves removed from pepper plants and (c) apparent uptake concentration (UC) of total N (total N uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum*, cv. ‘Sammy') grown in closed hydroponic systems. Vertical bars indicate ± standard errors of means.

The concentrations of SO_4 in the recirculating NS progressively increased with time when the Ca concentrations in the RNS were 3.0, 4.5, or 6.0 mmol L^{-1} Ca (Fig. 4.7a). However, when the Ca level in the RNS was 1.5 mmol L^{-1}, the Ca concentrations in the recirculating NS were stable throughout the whole experimental period. The concentrations of S in leaves were reduced at the flowering stage compared to the initial vegetative stage, irrespective of the applied treatments. Afterwards and until the end of the harvesting period, the concentrations of S in leaves increased following the SO_4 concentration differences in the RNS (Fig. 4.7b). The mean SO_4 UC increased with time in the medium and high Ca treatments, while in the low Ca treatment it remained initially constant and decreased by the latter cropping stages (Fig. 4.7c).

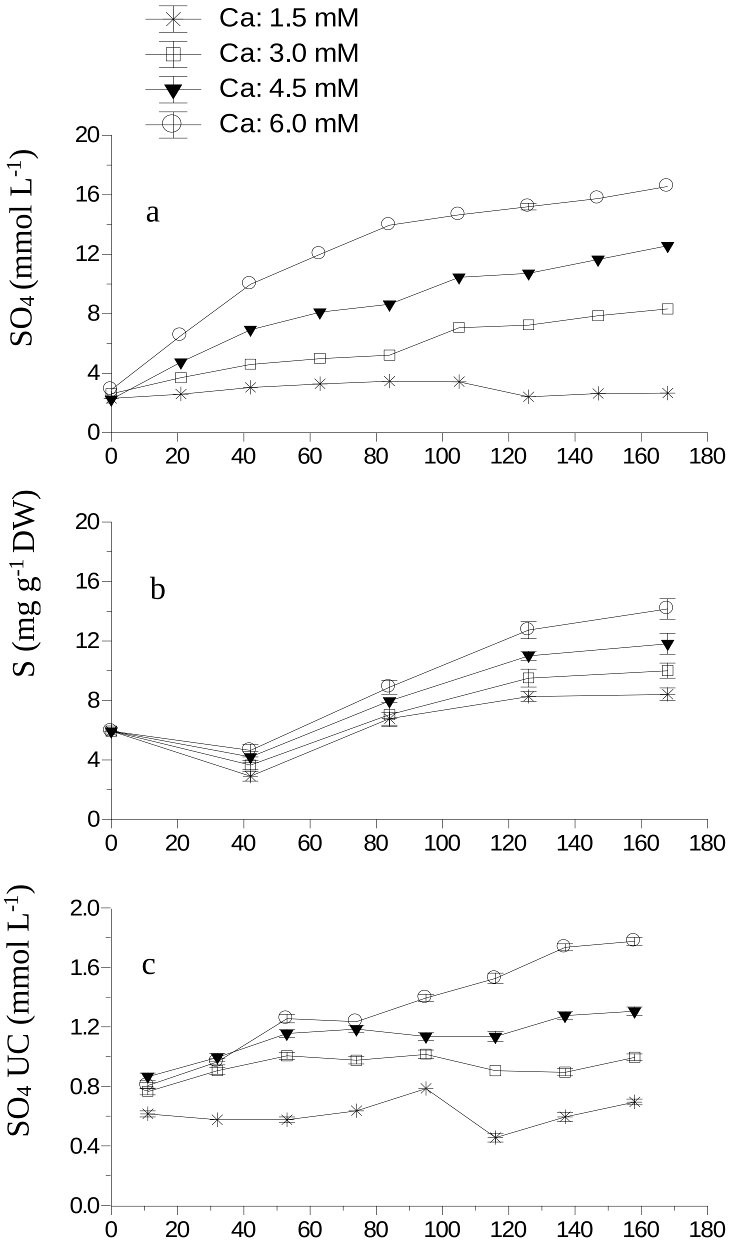


Figure 4.7. Concentrations: (a) of SO_4 in the recirculating nutrient solution (NS), (b) of S in the dry biomass (DW) of leaves removed from pepper plants and (c) apparent uptake concentration (UC) of SO_4 (SO_4 uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum*, cv. ‘Sammy') grown in closed hydroponic systems. Vertical bars indicate ± standard errors of means.

The evolution of K accumulation in the recycled solution with time is shown in Fig. 4.8a. Concentrations of K remained unaffected in all treatments until 40 DAT. Thereafter and until 84 DAT, the concentrations of K decreased in the NS supplied with 1.5 and 3.0 mM Ca, while in the other treatments (4.5 and 6 mM Ca) they remained unaffected. At 84 DAT, concentrations of K increased in all treatments while statistically significant differences among treatments were obtained. In specific, K concentration was lower in the control treatment, compared to all other treatments. Concentrations of K in leaves slightly increased until 84 DAT irrespective of the treatments. After the start of harvesting, concentrations of K decreased and remained unaffected by the applied treatments (Fig. 4.8b). Overall, the mean concentrations of K amounted to 70.7 mg (g DW)$^{-1}$ in leaves. The mean UC of K (7.0 mmol L^{-1}) did not differ significantly between treatments, while it remained at the same levels throughout the experimental period (Fig. 4.8c).

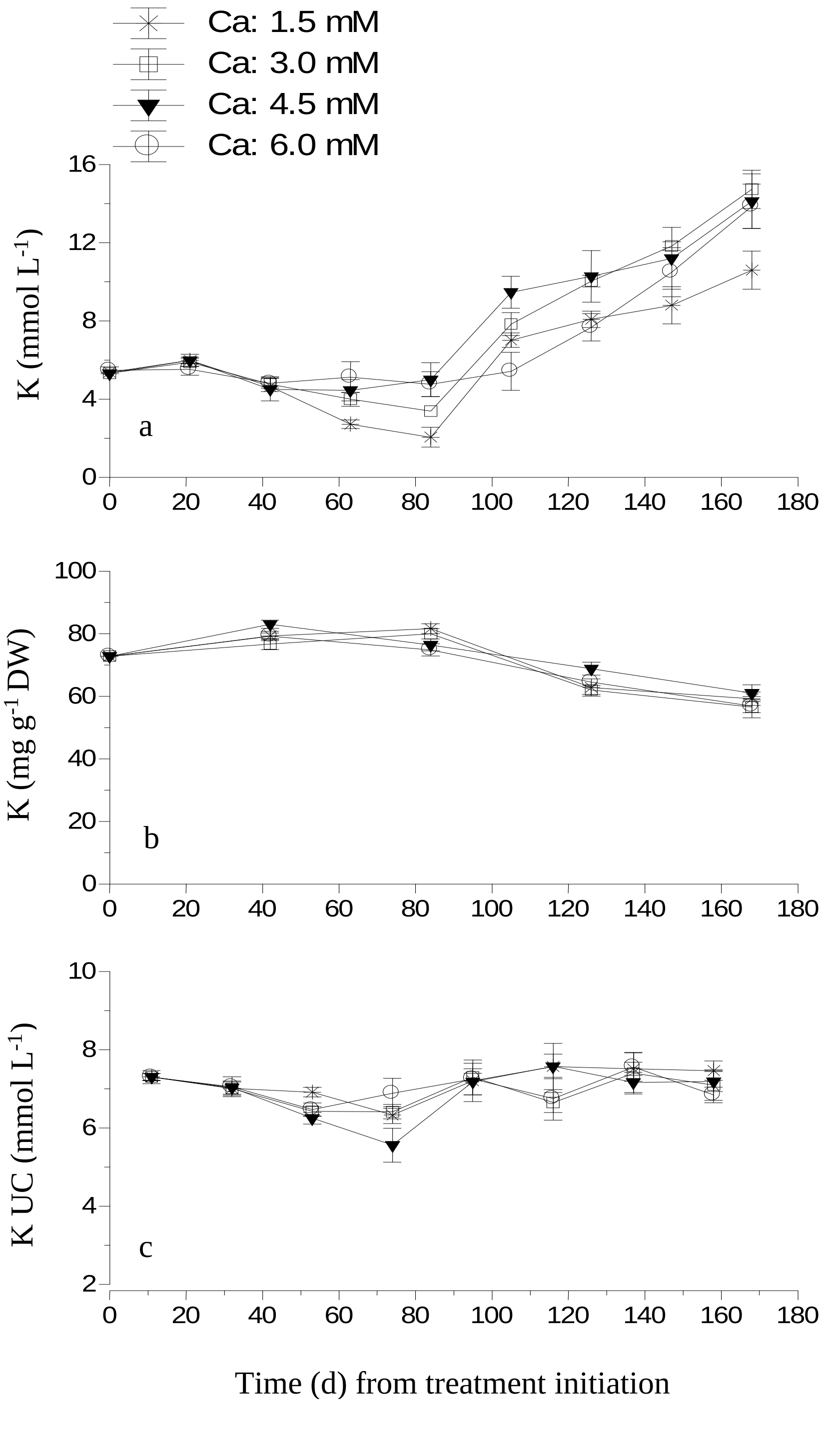


Figure 4.8 Concentrations of K: (a) in recirculating nutrient solution, (b) in the dry biomass (DW) of leaves and (c) apparent uptake concentration (UC) of K (K uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems. Vertical bars indicate ± standard errors of means

The concentrations of Mg increased during the flowering stage while they were not influenced by the applied treatments. However, during the first reproductive stage, Mg in the NS supplied with either 3.0, 4.5 or 6.0 mM Ca decreased and remained to these levels up to the end of the experiment. The Mg concentration was significantly lower when the Ca concentration in the RNS was lowest compared to the other three Ca-supply treatments (3.0, 4.5 or 6.0 mM Ca in the RNSs) (Fig. 4.9a).

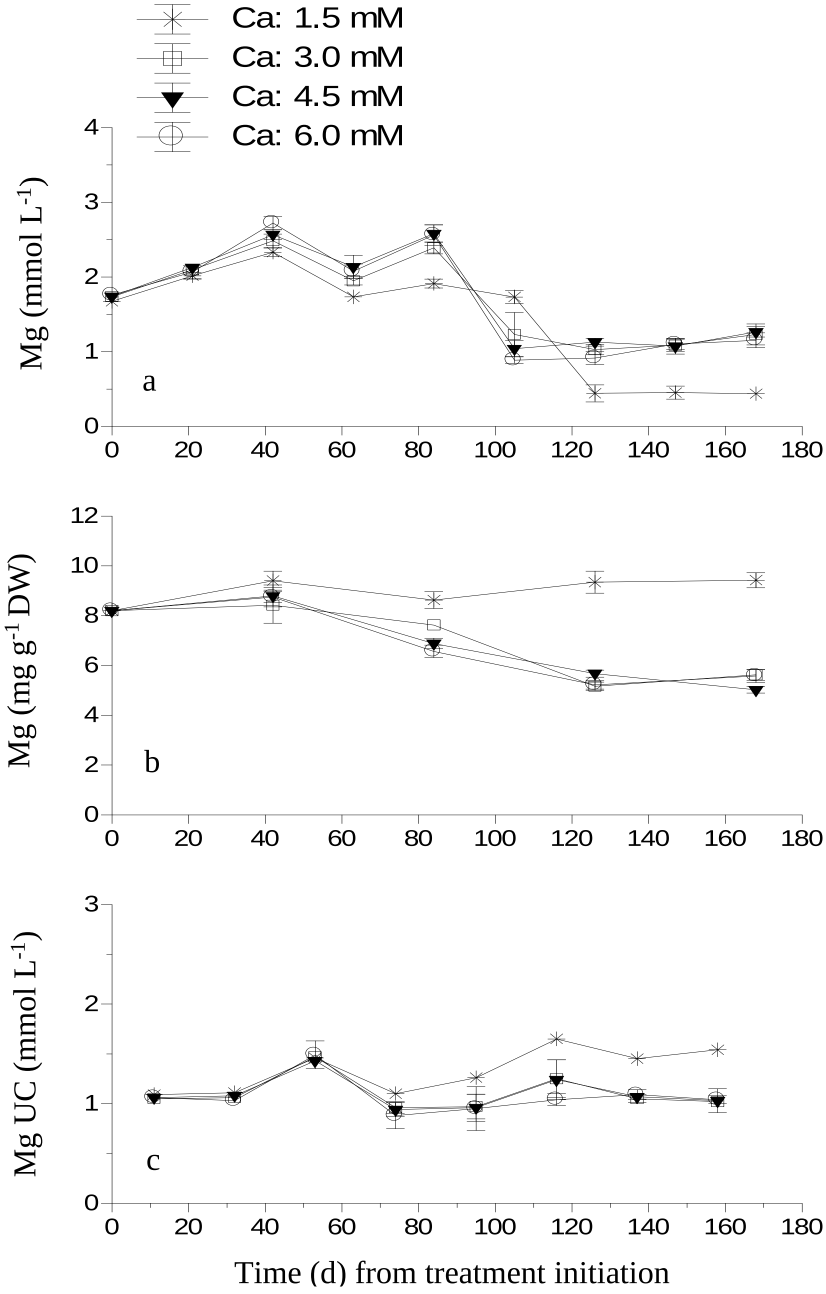


Figure 4.9. Concentrations of Mg: (a) in recirculating nutrient solution (NS), (b) in the dry biomass (DW) of leaves and (c) apparent uptake concentration (UC) of Mg (Mg uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems. Vertical bars indicate ± standard errors of means.

In the leaves of pepper plants, Mg levels did not change throughout the whole experimental period when plants were grown under control conditions (1.5 mM Ca in the RNS). In contrast, the Mg concentration in the leaves of all other treatments decreased with time, while no statistical differences were obtained between the treatments (Fig. 4.9b). The mean UC of Mg was also not influenced by the treatments until the start of harvesting. However, after that stage, the UC in the control treatment was significantly higher compared to that found in all other treatments (Fig. 4.9c).

4.5 Discussion

Pepper is considered sensitive to salinity (Rameshwaran et al 2016, Kpinkoun et al. 2018). Salinity induced by sodium chloride (NaCl) restricts the total fruit yield (Chartzoulakis & Klapaki 2000, Rubio et al. 2009). Maas and Hoffman (1977) estimated a salinity threshold value of 1.5 dS m^{-1} in the saturation extract for pepper plants grown in the soil and a yield decrease rate of 14% per unit (dS m^{-1}) increase of salinity above the threshold. Sonneveld and van der Burg (1991) also found a linear reduction of fruit yield at a rate of 7.6% as salinity increases above a threshold of 2.8 dS m^{-1} in the root-zone solution, when pepper is grown hydroponically and exposed to NaCl-salinity. The differences in the salinity threshold and the yield decrease rate due to salinity between these two studies is reasonable, since the EC in the saturation extract does not represent the actual EC in the soil solution but is approximately twice as high as under field capacity conditions (Neumann and Römheld 2012).

The data reported in the earlier-referenced studies were obtained by exposing the plants to soils or NS containing mainly NaCl in excess, while the current study addressed the responses of pepper to calcium-induced salinity. The number of studies aiming to assess the effect of calcium salinity on plant growth and nutrition is limited, although calcium bicarbonate ($Ca(HCO_3)_2$) is present at higher concentrations than NaCl in soils, groundwater and surface water in many areas worldwide (Biglari *et al*. 2016). The present study showed that salinity owing to excess Ca levels in the root zone is also harmful to pepper. Combined consideration of Fig. 4.2, 4.3 and 4.5 shows that a Ca concentration of 15 mmol L^{-1} in the root zone, imposed by accumulation when the Ca concentration in the RNS was 3 mmol L^{-1}, restrict yield by about 32%, but the decrease is not proportional to salinity increase. Indeed, a further increase of the Ca levels in the root zone to about 30 or 38 mmol L^{-1}, imposed by 4.5 or 6 mmol L^{-1} in the RNS, respectively, did not impose a further decrease yield.

Calcium is an essential plant macronutrient which fulfils fundamental functions associated with plant membrane stability, cell wall stabilization, and cell integrity (Hirschi 2004). However, calcium *per se* is not toxic to plants. Indeed, calcium concentrations in plant tissues can reach more than 10% in the dry weight without seriously inhibiting plant growth and development (Hawkesford *et al*. 2012). The damage caused by excessive external calcium concentrations is ascribed mainly to osmotic salinity effects (Huang *et al*. 2017) and competitive impairment of K, Mg and/or metallic micronutrient uptake (Falchi *et al.* 2017) which results in nutritional imbalances. Thus, the lack of a proportionality between yield reduction and increase of EC (and Ca levels) in the root zone of pepper seems to be associated with nutritional imbalances which apparently occurred even when the level of Ca in the NS surrounding the roots was 15 and 30 mM. It seems that the reduction of fruit production in these two treatments due to nutritional imbalances was similar to that caused by salinity in the highest Ca treatment, i.e. at 6.0 mM Ca in the supplied NS and about 35-40 mmol L^{-1} Ca in the recirculating NS. This hypothesis assumes that the damage caused by nutrient imbalances is not proportional to the level of the nutrient in excess that impairs the uptake of other nutrients, which is true according to Neocleous and Savvas (2018). Furthermore, this hypothesis assumes that the damage caused by impairment of nutrient uptake due to excess external Ca is not additive to that caused by high salinity. The reduction of the leaf Mg concentrations to similar levels in the medium and high Ca treatments (3.0, 4.5 and 6.0 mM) compared to 1.5 mM, irrespective of Ca level, is in agreement with this consideration.

In the current study, Ca-induced salinity restricted the fruit yield by reducing merely the number of fruit per plant, while the NaCl-induced salinity in the studies of Chartzoulakis and Klapaki (2000) and Navarro *et al.* (2002) impaired both the average fresh fruit weight and the number of fruits per plant. This finding further strengthens the notion that the harmful effects of calcium-salinity rely on different mechanisms than those imposed by NaCl-salinity.

In the current study, the moderate and high Ca concentrations (3.0, 4.5 and 6.0 mM) in the RNS raised the Ca concentrations in the recirculating solution to 17.2, 27.7 and 37.2 mmol L^{-1} respectively. As a result, the total salinity, expressed as EC, increased in the root zone at higher levels (6.4, 9.0 and 10.8 dS m^{-1}, respectively) than the recommended threshold of 2.8 dS m^{-1} according to Sonneveld and van der Burg (1991). This result indicates that the Ca UCs (mmol Ca per litre of water removed from the recirculating NS) in these treatments were lower than the Ca concentrations in the corresponding RNSs (mmol Ca per litre of water input). Indeed, as shown in Fig. 4.5c, the calculated Ca UCs in the moderate and high Ca treatments were lower than the Ca concentrations in the corresponding RNSs during most of the cropping period. Only for a short period before crop termination they exceeded slightly the Ca concentrations in the RNSs, and this imposed temporarily a small decrease of the Ca concentration in the recirculating solution. This is in agreement with the suggestion of Sonneveld and Voogt (2009) that the Ca concentration in the root environment of soilless-grown crops should be higher than the target Ca UC to ensure sufficient Ca uptake rates by the plants. A similar pattern with that of Ca, as shown in Fig. 4.5c, was found also for Na and Cl in a pepper crop grown in a closed hydroponic system (Savvas *et al*. 2007, Savvas *et al*. 2008).

The reduced water consumption in plants treated with 3.0, 4.5 and 6.0 mM Ca in the RNS (Fig. 4.1) compared to that recorded in plants treated with 1.5 mM Ca is because the Ca-stressed plants developed a less extensive leaf area than those treated with 1.5 mM Ca (data not shown), and thus the whole-plant transpiration was reduced. The reduced leaf area which points to a restriction of the vegetative growth in plants exposed to higher Ca concentrations than 15 mM in the root zone (i.e. 3 or higher than 3.0 mM Ca in the RNS) was presumably the causal factor for the lower fruit number per plant in these treatments. The restriction of the vegetative growth and yield to similar levels in the treatments with 3.0, 4.5 and 6.0 mM Ca in the RNS may be associated with the reduction of the leaf Mg concentration in these treatments, since the decrease in leaf Mg was also similar in these three Ca treatments compared to that measured in the low Ca treatment (1.5 mM in the RNS). Magnesium is a component of chlorophyll, while it is required for photosynthesis and protein synthesis (Hawkesford *et al.* 2012). As reported by Baszynski *et al.* (1980), Mg deficiency in plants leads to enhanced degradation of chlorophyll due to impaired export of photosynthates and accumulation of starch in chloroplasts which results to an increase of the dry matter content of the leaves. Thus, the reduced production of biomass in the medium and high Ca treatments may be associated with a partial impairment of the photosynthetic function. In addition, the accumulation of Ca can lead to osmotic stress as the plants strive to reduce their osmotic potential so that they can absorb water from the root environment despite the reduction of osmotic potential in the root environment due to increased Ca concentrations. The similar WUE in all treatments originates presumably from the similar reduction of fruit yield and water consumption by the Ca salinity.

The results illustrated in Fig. 4.5 show that the Ca UC (i.e. nutrient to water uptake ratio) was influenced by Ca concentration in the recirculating NS, which depends on the Ca concentration in the RNS (Neocleous and Savvas 2015), and the plant developmental stage. However, the impact of the plant developmental stage on the Ca UC is apparent only in the low Ca treatment (1.5 mM Ca in the RNS). This is because in the low Ca treatment, no Ca accumulation over time occurred, and thus the changes of the Ca UC over time were due only to the impact of the plant developmental stage and not due to Ca concentration changes in the external solution. As shown in Fig. 4.5c the Ca UC in the low Ca treatment is highest during the initial vegetative phase (2.6 mmol L^{-1}), i.e. during the first 30 DAT, while it decreases during the late vegetative stage, with a tendency to recover to an intermediate level (about 1.9 mmol L^{-1}) upon commencement of fruit production. In an experiment with melon, Neocleous and Savvas (2015) also found that the Ca UC was highest during the initial vegetative stage, i.e. during the first 30 DAT. The higher UCs during the initial vegetative stage are ascribed to the relatively low water requirements of young plants compared to their nutrient demand. Indeed, at the early developmental stage, the pepper plant does not bear large mature leaves, which normally exhibit high transpiration rates but low rates of net assimilation and concomitantly low nutrient requirements.

Unlike the Ca UC, the UC of total N were more stable over time (Fig. 4.6c) because, in contrast to Ca, total N enters the cells and accumulates to vacuoles to contribute to cell homeostasis acting as an energetically cheap osmoticum (MacRobbie 1976, Burns et al. 2012). Thus, the total N uptake rates do not decrease with time as the pepper plants develop many mature leaves. In tomato, the requirements for total N uptake decrease during the reproductive stage (Weerakkody *et al.* 2011), because the fruit needs less N than the leaves, but this does not seem to be the case with pepper. Adams (2002) also pointed out to the lower total N requirements of the acidic tomato fruit compared to those of the neutral pepper fruit.

Combined consideration of plates (a) and (c) in Fig. 4.5 indicates that the Ca concentration in the root zone was about 4 mmol L^{-1} when the Ca UC ranged from 1.5 to 2.3 mmol L^{-1}. It is well known that the ion concentrations in the root environment mostly differ from the corresponding UCs (Sonneveld 2002). Especially for bivalent ions such as Ca, Mg and SO_4, the concentrations in the root zone should be much higher that the corresponding UCs that are considered optimal for plant growth (Rameshwaran 2016). According to Sonneveld (2002) a Ca UC of 1.9 mmol L^{-1} requires a concentration of about 8 mmol L^{-1} in the root environment, which is similar to the values found in the current study. Higher external Ca concentrations in the current study imposed considerably higher Ca UCs, in agreement with relevant results reported for a zucchini squash crop (Neocleous and Savvas 2018). Another observation arising from the current study is that in the low Ca treatment, the Ca concentration in the recirculating NS tended to decrease with time and this imposed a commensurate decrease in the Ca UC. This finding indicates that a concentration of 1.5 mM in the RNS is not sufficient for pepper grown in closed hydroponic systems. Nevertheless, since a concentration of 3.0 mM in the RNS resulted in Ca accumulation, the optimal Ca concentration in RNS for pepper grown in closed hydroponic systems under Mediterranean climatic conditions should be higher than 1.5 mM but lower than 3.0 mM, and closer to 1.5 rather than to 3.0 mM.

In the current study, the increase of the Ca concentration in the RNS supplied to the different treatments was electrochemically balanced by commensurate increases in the NO_3 and SO_4 concentrations. However, the increase of the NO_3 and SO_4 concentrations from 11.5 and 0.7 mmol L^{-1}, respectively, in the RNS to 13.5 and 1.2 mmol L^{-1} or higher, respectively, resulted in gradual accumulation of their concentrations in the recirculating NS, which is not desirable. Thus, the NO_3 and SO_4 concentrations in the RNS supplied to pepper grown in closed hydroponic systems should be lower than 13.5 and 1.2 mmol L^{-1}, respectively. Nevertheless, the NO_3 and SO_4 concentrations in the RNS supplied to the low Ca treatment (11.5 and 0.7 mmol L^{-1}), gradually resulted in decreasing levels of NO_3 and SO_4 in the recirculating NS, which is also not desirable. Thus, the results of the current study indicate that the optimal NO_3 and SO_4 concentrations in the RNS supplied to pepper crops cultivated in closed hydroponic systems are in the range 11.5-13.5 mmol L^{-1} for NO_3 and 0.7-1.2 mmol L^{-1} for SO_4. These ranges are in agreement with the NO_3 and SO_4 concentrations suggested by Sonneveld and Voogt (2009) for RNS supplied to pepper in closed soilless systems (12.75 mmol L^{-1} for NO_3 and 1 mmol L^{-1} for SO_4). Nevertheless, more targeted research is needed to corroborate these ranges under different calcium concentrations.

4.6 Conclusion

In the current study, a Ca concentration of 3.0 mmol L^{-1} in the RNS, which resulted in calcium accumulation up to 17 mmol L^{-1} and an EC of 6.4 dS m^{-1} in the recirculating NS, restricted fruit yield by 22% compared to 1,5 mmol L^{-1} in the RNS, which did not impose any Ca accumulation in the recirculating NS. However, the further accumulation of Ca to 28 and 37 mmol L^{-1} in the recycled NS did not decrease significantly the total fruit yield, compared to 17 mmol L^{-1}. This result indicates that the yield reduction imposed by Ca accumulation in the recirculating NS originates mainly from nutrient imbalances caused by cation competition in uptake rather than from osmotic salinity effects, which normally decrease yield linearly in relation to salinity increase. The mean uptake ratios between nutrients (i.e. total N, SO_4, K, Ca and Mg) and water, denoted as UCs, were determined within a wide range of Ca concentrations in the root zone and could be used as a sound basis to establish standard RNS compositions for closed hydroponic crops of pepper under

Mediterranean climatic conditions. However, water resources with Ca concentrations of 3.0 mmol L^{-1} or higher, cannot be safely used in closed hydroponic crops of pepper as they raise salinity to harmful levels for fruit production. Furthermore, the current study indicated that the optimal NO_3 and SO_4 concentrations in RNS supplied to closed hydroponic crops of pepper should range between 11.5–13.5 and 0.7– 1.2 mmol L^{-1}, respectively, to avoid their accumulation in the root zone.

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Supplementary Tables

Table S1a. Statistical significance of the differences in water consumption (CWC) in a greenhouse crop of pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems as influenced by four different concentrations of Ca in the RNS. In each date different letters indicates significant differences between means (n=5) according to Duncan's Multiple Range Test.

Water consumption (CWC)									
Days / mM Ca	0	21	42	63	84	105	126	147	168
1.5	NS	NS	NS	NS	a	a	a	a	a
3.0					ab	ab	b	b	b
4.5					b	b	b	b	b
6.0					b	b	b	b	b

Table S1b. Statistical significance of the differences in Electrical conductivity (EC) in drainage water in a greenhouse crop of pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems as influenced by four different concentrations of Ca in the RNS. In each date different letters indicate significant differences between means (n=5) according to the Duncan's Multiple Range Test.

Electrical conductivity (EC)									
Days/ mM Ca	0	21	42	63	84	105	126	147	168
1.5	NS	b	d	d	d	d	d	d	d
3.0		ab	c	c	c	c	c	c	c
4.5		ab	b	b	b	b	b	b	b
6.0		a	a	a	a	a	a	a	a

Table S1c. Statistical significance of the differences in the concentrations of Ca: (A) in recirculating nutrient solution (NS), (B) in the dry biomass (DW) of leaves removed from pepper plants and (C) apparent uptake concentration (UC) of Ca (Ca uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems. In each date different letters indicates significant differences between means (n=5) according to the Duncan's Multiple Range Test.

(A) Concentrations of Ca in recirculating nutrient solution (NS)									
Days/ mM Ca	0	21	42	63	84	105	126	147	168
1.5	NS	c	d	d	d	d	d	d	d
3.0		b	c	c	c	c	c	c	c
4.5		b	b	b	b	b	b	b	b
6.0		a	a	a	a	a	a	a	a

(B) Concentrations of Ca in the dry biomass (DW) of leaves removed from pepper plants					
Days/ mM Ca	0	42	84	126	168
1.5	NS	b	b	b	c
3.0		b	a	b	c
4.5		a	a	a	b
6.0		a	a	a	a

(C) Apparent uptake concentration (UC) of Ca (Ca uptake per water uptake)								
Days/ mM Ca	11	32	53	74	95	116	137	158
1.5	d	b	c	c	c	d	d	d
3.0	c	ab	c	b	b	c	c	c
4.5	b	ab	b	b	b	b	b	b
6.0	a	a	a	a	a	a	a	a

Table S1d. Statistical significance of the differences in the concentrations of total N ($NO_3 + NO_4$): (A) in recirculating nutrient solution (NS), (B) in the dry biomass (DW) of leaves and (C) apparent uptake concentration (UC) of total N (total N uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems. In each date different letters indicates significant differences between means (n=5) according to the Duncan's Multiple Range Test.

(A) Concentrations of total N in recirculating nutrient solution (NS)									
Days/ mM Ca	0	21	42	63	84	105	126	147	168
1.5	NS	d	d	d	d	d	d	d	d
3.0		c	c	c	c	c	c	c	c
4.5		b	b	b	b	b	b	b	b
6.0		a	a	a	a	a	a	a	a

(B) Concentrations of total N in the dry biomass (DW) of leaves removed from pepper plants					
Days/ mM Ca	0	42	84	126	168
1.5	NS	c	NS	ab	ab
3.0		b		ab	b
4.5		a		b	b
6.0		a		a	a

(C) Apparent uptake concentration (UC) of total N (total N uptake per water uptake)								
Days/ mM Ca	11	32	53	74	95	116	137	158
1.5	d	d	d	d	d	d	d	d
3.0	c	c	c	c	c	c	c	c
4.5	b	b	b	b	b	b	b	b
6.0	a	a	a	a	a	a	a	a

Table S1e. Statistical significance of the differences in the concentrations: (A) of SO_4 in the recirculating nutrient solution, (B) of S in the dry biomass (DW) of leaves and (C) apparent uptake concentration (UC) of SO_4 (SO_4 uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum*, cv. 'Sammy') grown in closed hydroponic systems. In each date different letters indicates significant differences between means (n=5) according to the Duncan's Multiple Range Test.

(A) Concentrations of SO_4 in recirculating nutrient solution (NS)									
Days/ mM Ca	0	21	42	63	84	105	126	147	168
1.5	NS	d	d	d	d	d	d	d	d
3.0		c	c	c	c	c	c	c	c
4.5		b	b	b	b	b	b	b	b
6.0		a	a	a	a	a	a	a	a

(B) Concentrations of S in the dry biomass (DW) of leaves removed from pepper plants					
Days/ mM Ca	0	42	84	126	168
1.5	NS	c	c	d	d
3.0		bc	c	c	c
4.5		ab	b	b	b
6.0		a	a	a	a

(C) Apparent uptake concentration (UC) of SO_4 (SO_4 uptake per water uptake)								
Days/ mM Ca	11	32	53	74	95	116	137	158
1.5	c	c	d	c	d	d	d	d
3.0	ab	b	c	c	c	c	c	c
4.5	a	a	b	b	b	b	b	b
6.0	ab	a	a	a	a	a	a	a

Table S1f. Statistical significance of the differences in the concentrations: (A) of K in recirculating nutrient solution (NS), (B) of K in the dry biomass (DW) of leaves and (C) apparent uptake concentration (UC) of K (K uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum,* cv. ‘Sammy') grown in closed hydroponic systems. In each date different letters indicates significant differences between means (n=5) according to the Duncan’s Multiple Range Test.

(A) Concentrations of K in recirculating nutrient solution (NS)									
Days/ mM Ca	0	21	42	63	84	105	126	147	168
1.5	NS	NS	NS	c	c	b	b	c	b
3.0				b	b	b	a	a	a
4.5				ab	a	a	a	ab	a
6.0				a	a	c	b	bc	a

(B) Concentrations of K in the dry biomass (DW) of leaves removed from pepper plants					
Days/ mM Ca	0	42	84	126	168
1.5	NS	NS	NS	NS	NS
3.0					
4.5					
6.0					

(C) Apparent uptake concentration (UC) of K (K uptake per water uptake)								
Days/ mM Ca	11	32	53	74	95	116	137	158
1.5	NS	NS	a	a	NS	a	NS	NS
3.0			b	a		b		
4.5			b	b		ab		
6.0			b	a		b		

Table S1g. Statistical significance of the differences in the concentrations: (A) of Mg in recirculating nutrient solution (NS), (B) of Mg in the dry biomass (DW) of leaves and (C) apparent uptake concentration (UC) of Mg (Mg uptake per water uptake), as influenced by four different Ca concentrations in the RNS supplied to pepper (*Capsicum annum,* cv. 'Sammy') grown in closed hydroponic systems. In each date different letters indicates significant differences between means (n=5) according to the Duncan's Multiple Range Test.

(A) Concentrations of Mg in recirculating nutrient solution (NS)									
Days/ mM Ca	0	21	42	63	84	105	126	147	168
1.5	NS	NS	b	b	b	a	b	b	b
3.0			ab	a	a	b	a	a	a
4.5			ab	a	a	b	a	a	a
6.0			a	a	a	b	a	a	a

(B) Concentrations of Mg in the dry biomass (DW) of leaves removed from pepper plants					
Days/ mM Ca	0	42	84	126	168
1.5	NS	NS	a	a	a
3.0			b	b	b
4.5			c	b	b
6.0			c	b	b

(C) Apparent uptake concentration (UC) of Mg (Mg uptake per water uptake)									
Days/ mM Ca	11	32	53	74	95	116	137	158
1.5	NS	NS	NS	a	a	a	a	a
3.0				b	b	b	b	b
4.5				b	b	bc	b	b
6.0				b	b	c	b	b

Supplementary Figure

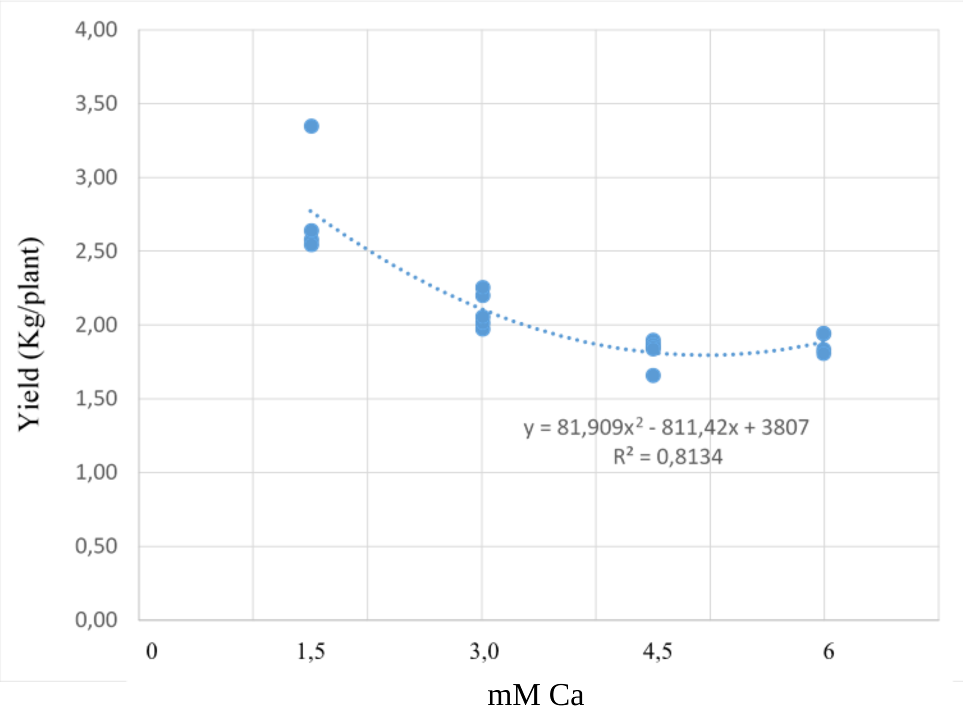

Figure S4. Yield (kg/plant) in a greenhouse crop of pepper (*Capsicum annum*, cv. ‘Sammy') grown in closed hydroponic systems as influenced by four different concentrations of Ca in the RNS

CHAPTER 5. GENERAL DISCUSSION

In the first study, in autumn-spring cultivation, both the water consumption and the water use efficiency were suppressed by the low temperature regime but the temperature effect interacted with the rootstock/scion combination. Indeed, grafting ‘Orangery’ onto ‘Robusto’ had no impact on WUE because it had no impact on both fruit yield and water consumption. However, grafting ‘Orangery’ onto ‘Terrano’ had no impact on WUE because this rootstock/scion combination increased proportionally both the fruit yield and the water consumption as it enhanced the vegetative biomass production. Furthermore, grafting ‘Sammy’ increased the WUE irrespective of the tested rootstocks because both ‘Robusto’ and ‘Terrano’ increased yield without affecting the vegetative plant biomass and concomitantly the water consumption via transpiration. Colla *et al*. (2008) and López-Marí*n et al.* (2013) also found that grafting different scions onto the same rootstock and vice versa may result in different yield responses.

The results in the first study showed that yield decreases imposed by low temperature is an effect that were fully anticipated, since pepper is a warm-season crop with a high susceptibility to suboptimal temperatures (Airaki *et al.* 2012). The yield decrease by about 50% in ‘Sammy’ and 33% in ‘Orangery’ imposed by low temperature, irrespective of the grafting combination, resulted from a reduction of the mean fruit weight (MFW) in ‘Orangery’ and from a reduction of both fruit number per plant (TFN) and MFW in ‘Sammy’. Moreover, the suppressive effect of low temperatures on pepper yield did not interact with grafting and the rootstock, which indicates that rootstocks 'Robusto' and 'Terrano' have no impact on cold tolerance of pepper. However, previous research has indicated that the tolerance to low temperature in pepper depends not only on the rootstock genotype but on the rootstock/scion combination (Shu *et al.* 2016). Both tested rootstocks 'Robusto' and 'Terrano' increased the total fruit yield by 39% and 34% compared with the self-grafted control when the scion was the commercial variety ‘Sammy’. However, when the scion was ‘Orangery’, only ‘Terrano’ was capable of increasing the total fruit yield by 35% compared with that obtained from self-grafted ‘Orangery’ plants, while ‘Robusto’ had no impact on yield. The results of the present study showed that the impact of grafting on fruit yield is depending on the rootstock/scion combination, which is in agreement with the suggestion of Rouphael *et al.* (2017), López-Marín *et al.* (2013) and Savvas *et al.* (2010). In ‘Sammy’, the absence of any impact of grafting on total plant biomass and water consumption despite the significant increase in the fruit number per plant points to a positive effect of both tested rootstocks on fruit setting rather than on plant vigor. A similar response was observed also by López-Marín *et al.* (2013), with the sweet pepper cultivar Herminio grafted onto the commercial rootstock Creonte. On the other hand, when ‘Orangery was grafted onto ‘Terrano’ in the present study, both the plant biomass and the water consumption increased proportionally to the increase in fruit number per plant, which indicates that this grafting combination increases yield due to stimulation of plant vigor. However, in this study, all grafting combinations increased yield exclusively by raising the number of fruit per plant, while they had no effect on the mean fruit weight. Colla *et al.* (2008), who grafted the sweet pepper cultivar ‘Edo’ onto five different rootstocks, found that four of them increased significantly the total fruit yield and in all cases the yield increase was exclusively due to a higher fruit number per plant.

The third study showed that, in autumn-winter cultivation, the reduced water consumption in ‘Sammy’ treated with 3.0, 4.5 and 6.0 mM Ca in the RNS compared to that recorded in plants treated with 1.5 mM Ca, is because the Ca-stressed plants developed a less extensive leaf area than those treated with 1.5 mM Ca and thus the whole-plant transpiration was reduced. The reduced leaf area which points to a restriction of the vegetative growth in plants exposed to higher Ca concentrations than 15 mM in the root zone (i.e. 3 or higher than 3.0 mM Ca in the RNS), was presumably the causal factor for the lower fruit number per plant in these treatments. The restriction of the vegetative growth and yield to similar levels in the treatments with 3.0, 4.5 and 6.0 mM Ca in the RNS may be associated with the reduction of the leaf Mg concentration in these treatments, since the decrease in leaf Mg was also similar in these three Ca treatments compared to that measured in the low Ca treatment (1.5 mM in the RNS). Magnesium is a component of chlorophyll, while it is required for photosynthesis and protein synthesis (Hawkesford *et al.* 2012). As reported by Baszynski *et al.* (1980), Mg deficiency in plants leads to enhanced degradation of chlorophyll due to impaired export of photosynthates and accumulation of starch in chloroplasts which results to an increase of the dry matter content of the leaves. Thus, the reduced production of biomass in the medium and high Ca treatments may be associated with a partial impairment of the photosynthetic function. In addition, the accumulation of Ca can lead to osmotic stress as the plants strive to reduce their osmotic potential so that they can absorb water from the root environment despite the reduction of osmotic potential in the root environment due to increased Ca concentrations. The similar WUE in all treatments originates presumably from the similar reduction of fruit yield and water consumption by the Ca salinity. In this study, Ca-induced salinity restricted the fruit yield by reducing merely the number of fruit per plant, while the NaCl-induced salinity in the studies of Chartzoulakis and Klapaki (2000) and Navarro *et al.* (2002) impaired both the average fresh fruit weight and the number of fruits per plant. This finding further strengthens the notion that the harmful effects of calcium-salinity rely on different mechanisms than those imposed by NaCl-salinity. The damage caused by excessive external calcium concentrations is ascribed mainly to osmotic salinity effects (Huang *et al*. 2017) and competitive impairment of K, Mg and/or metallic micronutrient uptake (Falchi *et al.* 2017) which results in nutritional imbalances. The present study showed that salinity owing to excess Ca levels in the root zone is also harmful to pepper. Indeed, Ca concentration of 15 mmol L^{-1} in the root zone, imposed by accumulation when the Ca concentration in the RNS was 3 mmol L^{-1}, restrict yield by about 32%, but the decrease is not proportional to salinity increase. Thus, a further increase of the Ca levels in the root zone to about 30 or 38 mmol L^{-1}, imposed by 4.5 or 6 mmol L^{-1} in the RNS, respectively, did not impose a further decrease yield.

Certain grafting combinations may restrict the uptake of some micronutrients and non-nutrient ions, an effect that may be helpful to crops exposed to excessive concentrations of these ions in the root zone, as reported by Savvas *et al*. (2010) and Rouphael *et al.* (2017). In the first study, both ‘Robusto’ and ‘Terrano’ reduced slightly, but significantly, the Mn uptake by ‘Orangery’, while the same rootstocks had no impact on Mn uptake when the scion was ‘Sammy’. In the second study also found no impact of grafting on Mn uptake concentrations. The different responses of ‘Sammy’ and ‘Orangery’ to grafting concerning the Mn uptake further support the notion that the impact of grafting on nutrient uptake depends on the specific rootstock/scion combination rather than on the rootstock genotype.

In the first study, the increases in the UCs of K, Ca, Mg and N under low temperature conditions was a consequence of the reduction in water consumption by 24%. Especially the increases in the uptake concentrations of Ca (22%) and Mg (27%) were very similar to the reduction in water consumption, which indicates that the absolute amounts of Ca and Mg absorbed by the crop were not restricted by low temperature, despite the reduction in plant biomass. Unlike the uptake concentrations of metallic micronutrient cations, that of P was reduced by low temperature. These results indicate that the uptake mechanism of $H_2PO_4^-$ by pepper is much more susceptible to low temperature than that of K, Ca, Mg and N. As shown by Adams (1994), the uptake of P responds much stronger to the root temperature than the uptake of N and K. The low temperature treatments in the present study resulted in low temperatures also in the root zone, which recovered slower during the day than the air temperature (data not shown). Phosphorus occurs at much lower concentrations in the soil solution compared to other macronutrients (Bieleski 1973). Therefore, during their evolution, plants developed an active uptake mechanism for P uptake, which is much more depending on the availability of metabolic energy in the roots than for uptake of other nutrients (Schachtman *et al*. 1998). However, the low root temperature decreases root respiration and consequently the availability of metabolic energy for active P uptake (Sutton 1969*)*. Thus, the profound reduction of P uptake by pepper in the LT regime may be ascribed mainly to the low root temperature. From another point of view, the results of the present study provide support to the notion that the uptake of P by pepper is stronger influenced by root temperature than by that prevailing in the air.

The average UCs found during the vegetative cropping period under ST conditions were 2.92 mmol L^{-1} for Ca, 0.92 mmol L^{-1} for Mg, 6.03 mmol L^{-1} for K, 12.14 mmol L^{-1} for N, and 0.89 mmol L^{-1} for P. Furthermore, the UC of macronutrients estimated in the second study ranged from 2.4 to 3.7 mmol L^{-1} for Ca, 1.0 to 1.5 mmol L^{-1} for Mg, 6.2 to 9.0 mmol L^{-1} for K, 11.7 to 13.7 mmol L^{-1} for N, and 0.7 to 1.1 mmol L^{-1} for P. Finally, the UCs found by Voogt and Sonneveld (1997) in a pepper crop grown in a closed rockwool system in the Netherlands, averaged for the whole cropping period, were 2.2 mmol L^{-1} for Ca, 0.78 mmol L^{-1} for Mg, 4.6 mmol L^{-1} for K, 10.30 mmol L^{-1} for N, and 0.81 mmol L^{-1} for P. Comparing the data from these three different crops shows that the UC of Ca, Mg, K, and N are generally higher in winter crops of pepper grown under Mediterranean climatic conditions than those reported for Dutch greenhouses, while that of P is similar in both environments. The values found in the second study are comparable with those found in the first study, although Mg and K tended to be somewhat lower in the first study. Both the first and second study showed that the temperature inside the greenhouse, the cultivar, and the rootstock when using grafted plants, may have a significant impact on the UCs. Since these factors may vary in different crops, the UCs found in the three studies are only indicative. Therefore, their use as a basis to prepare nutrient solutions for closed hydroponic systems entails frequent readjustment following measurements of the nutrient concentrations in the root zone of the crop, as suggested by Savvas and Gruda (2018).

In the third study, the Ca concentration in the root zone was about 4 mmol L^{-1} when the Ca UC ranged from 1.5 to 2.3 mmol L^{-1}. According to Sonneveld (2002) a Ca UC of 1.9 mmol L^{-1} requires a concentration of about 8 mmol L^{-1} in the root environment, which is similar to the values found in the current study. Higher external Ca concentrations in the current study imposed considerably higher Ca UCs, in agreement with relevant results reported for a zucchini squash crop (Neocleous and Savvas 2018). Another observation arising from this study is that a concentration of 1.5 mM in the RNS is not sufficient for pepper grown in closed hydroponic systems, since the Ca concentration in the recirculating NS tended to decrease with time and this imposed a commensurate decrease in the Ca UC. Nevertheless, since a concentration of 3.0 mM in the RNS resulted in Ca accumulation, the optimal Ca concentration in RNS for pepper grown in closed hydroponic systems under Mediterranean climatic conditions should be higher than 1.5 mM but lower than 3.0 mM. Furthermore, the increase of the Ca concentration in the RNS supplied to the different treatments was electrochemically balanced by commensurate increases in the NO_3 and SO_4 concentrations. However, the increase of the NO_3 and SO_4 concentrations from 11.5 and 0.7 mmol L^{-1}, respectively, in the RNS to 13.5 and 1.2 mmol L^{-1} or higher, respectively, resulted in gradual accumulation of their concentrations in the recirculating NS, which is not desirable. Thus, the NO_3 and SO_4 concentrations in the RNS supplied to pepper grown in closed hydroponic systems should be lower than 13.5 and 1.2 mmol L^{-1}, respectively. Nevertheless, the NO_3 and SO_4 concentrations in the RNS supplied to the low Ca treatment (11.5 and 0.7 mmol L^{-1}), gradually resulted in decreasing levels of NO_3 and SO_4 in the recirculating NS, which is also not desirable. Thus, the results of the current study indicate that the optimal NO_3 and SO_4 concentrations in the RNS supplied to pepper crops cultivated in closed hydroponic systems are in the range 11.5-13.5 mmol L^{-1} for NO_3 and 0.7-1.2 mmol L^{-1} for SO_4. These ranges are in agreement with the NO_3 and SO_4 concentrations suggested by Sonneveld and Voogt (2009) for RNS supplied to pepper in closed soilless systems (12.75 mmol L^{-1} for NO_3 and 1 mmol L^{-1} for SO_4). Nevertheless, more targeted research is needed to corroborate these ranges under different calcium concentrations.

CHAPTER 6. GENERAL CONCLUSIONS

The results of the first study indicated that minimizing greenhouse heating under Mediterranean climatic conditions so as to maintain the minimum inside day/night temperature at 12/7 °C, respectively, suppressed the fruit yield of pepper to levels depending on the cultivar, but not on grafting onto 'Robusto' or 'Terrano'. The yield decrease imposed by low temperature (LT) resulted from a reduction of the mean fruit weight (MFW) in ‘Sammy’ and from a reduction of both fruit number per plant (TFN) and MFW in ‘Orangery’. Grafting of ‘Sammy’ onto both 'Robusto' and 'Terrano' increased the fruit yield, while grafting of ‘Orangery’ increased yield only when the rootstock was ‘Terrano’. The yield increase imposed by grafting resulted exclusively from enhancement of the fruit number per plant (TFN). The water use efficiency (WUE) decreased considerably at LT, irrespective of the rootstock/scion combination. Grafting of ‘Sammy’ increased significantly the WUE irrespective of the scion at LT, but only when the rootstock was ‘Robusto’ at ST. Grafting of ‘Orangery’ had no impact on WUE, irrespective of the rootstock and the temperature regime. The LT increased the uptake concentrations (UC) of K, Ca, Mg, N, and Mn, while it decreased strongly that of P and slightly those of Fe and Zn. The UC of K and Mg were influenced by the rootstock/scion combination but this effect interacted with the temperature regime. In contrast, the Ca, N, and P concentrations were not influenced by the grafting combination. Furthermore, the impact of grafting on yield and nutrient uptake in pepper depend not merely on the rootstock genotype but on the rootstock/scion combination. Finally, the first study indicated that under ST Mediterranean climatic conditions in a winter hydroponical cultivation of sweet pepper, the average UCs during the vegetative cropping period were 2.92 mmol L^{-1} for Ca, 0.92 mmol L^{-1} for Mg, 6.03 mmol L^{-1} for K, 12.14 mmol L^{-1} for N, and 0.89 mmol L^{-1} for P.

The results of the second study showed that different pepper cultivars may take up nutrients and water at different ratios under the same nutritional, irrigation and climatic Mediterranean conditions, as indicated by the observed differences in the UC. More specifically, ‘Sondela’ exhibited the highest Mg and Ca UC throughout the cropping period and B UC during the reproductive stage in comparison with all other tested cultivars. Furthermore, ‘Belissa’ exhibited significantly higher K UC throughout the cropping period in comparison with all other tested cultivars. The tissue nutrient concentrations and the UC were similar in ‘Sammy’ self-grafted and ‘Sammy’ grafted onto the commercial rootstock `RS10' (*Capsicum annuum*), which indicates that this *Capsicum annum* rootstock does not modify the uptake of nutrients and water by the scion. The developmental stage of the pepper plants had a strong impact on the UCs of most nutrients due to changes in the mean physiological age of the leaves, the onset of fruit production and seasonal differences in climatic conditions. Based on the UCs estimated in the first study the nutrient solutions supplied to closed hydroponic crops of pepper should contain Ca, Mg and B at higher concentrations than standard recommendations when the cultivated variety is ‘Sondela’ and K at higher concentrations when the cultivated variety is ‘Bellisa’. The UCs of macronutrients under ST conditions in the second study ranged from 2.4 to 3.7 mmol L^{-1} for Ca, 1.0 to 1.5 mmol L^{-1} for Mg, 6.2 to 9.0 mmol L^{-1} for K, 11.7 to 13.7 mmol L^{-1} for N, and 0.7 to 1.1 mmol L^{-1} for P. Comparing the data from the UCs of macronutrients in the first and the second study with the UCs found by Voogt and Sonneveld (1997), these three different crops shows that the UC of Ca, Mg, K, and N are generally higher in winter crops of pepper grown under Mediterranean ST climatic conditions than those reported for Dutch greenhouses, while that of P is similar in both environments. Both the first study and in the second study, showed that the temperature inside the greenhouse, the cultivar, and the rootstock when using grafted plants, may have a significant impact on the UCs.

The results of the third study, in an autumn-winter closed hydroponic crop of pepper under Mediterranean climatic conditions, showed that calcium salinity in pepper cultivar ‘Sammy’ decreased gradually yield at salinity level in root zone above of 1.5 dS/m, which is equal with the salinity threshold estimated by Maas and Hoffman (1977). The mean uptake ratios of nutrients (i.e., N, S, K, Ca and Mg) and water were determined within a wide range of Ca concentrations in the root zone and could be used as a rough basis for the establishment of nutrient solution compositions for closed pepper crops under Mediterranean climates. However, water resources containing Ca concentrations higher than 3.0 mM cannot be safely used in closed hydroponic crops of pepper (raised Ca level up in the root zone to 6.4 mM) in terms of growth and yield of the produce, especially if there is excess of salts in the soil solution or/and in the irrigation water. The above results may be used as a tool in modelling the crop-recirculation system, in areas where low quality water is usually used for irrigation. Furthermore, more targeted research is needed to corroborate these ranges under different calcium concentrations. This study is useful for plant producers by proposing tests for screening new or existing hybrids for their tolerance to salt. The UCs found in the three studies are only indicative and further research is needed since these factors may vary in different crops.

Curriculum vitae

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Education:

1990: Lyceum of Itea, Fokida.

1999: Bachelor of Science (BSc) in Agronomy, Agricultural University of Athens. Department of Crop Science, Laboratory of Vegetable Production, (The program is a five-year curriculum), Athens.

2011: Master in Education (M.Ed.), Hellenic Open University, (The program is a three-year curriculum), Patra.

2021: Ph.D. Dissertation, University of Thessaly, Department of Agriculture Crop Production and Rural Environment, Volos.

Professional Experience:

1999: Practical work at Agricultural Research Station of Arta.

2000: Working in the Office of Agricultural Development - Department of Agriculture & Livestock in Amfissa as an Agronomist.

2002: Working in the Union of Amfissa's Agricultural Cooperatives in Amfissa as an Agronomist.

2002-3: Working in the Society of Social Psychiatry & Mental Health in Amfissa as an Agronomist.

2003-14: Technical and Laboratory Staff at the Agricultural University of Athens, Department of Crop Science, Laboratory of Vegetable Production.

2014-now: Teaching Assistant at the Agricultural University of Athens, Department of Crop Science, Laboratory of Vegetable Production.

Languages

2012: Michigan Proficiency (CPE) in English.

Computer Skills

2000: MS-DOS, Microsoft Access, Microsoft Word, Microsoft Excel (Διεθνές δίκτυο AXON).

Recent research projects:

1. Member of the scientific team of the Agricultural University of Athens in a Project titled «Development of good agricultural practices in horticulture in pumice and diffusion of the results in the cultivation practice» and ELKE code 34.0231, funded by LAVA A.E.. Duration: 01/04/2011 – 29/02/2012 and 01/03/2012 - 31/12/2012.

2. Member of the research team in a Research Project of the action ARCHIMEDES, which was co-funded by the EU and the Greek Ministry of Education and Religions titled: «Effect of mycorhizal and other symbiotic microorganisms on plants cultivated in soil and soilless culture systems under biotic and abiotic stress conditions" which is implemented by the Faculty of Agricultural technology of TEI of Epirus Duration of the project: 01/01/2013 to 31/12/2015.
3. Member of the research team in in the European Research Program of Horizon 2020 (H2020) which is prepared by the Vegetable Laboratory of the Agricultural University of Athens entitled: "TRUE: Transition paths to sustainable legume-based systems in Europe (EU Code: 727973) and scientific supervisor Professor D. Savva. Project duration: 01/05/2017 – 31/12/2017.

Publications

I. Publications in international refereed journals with impact factor

1. Savvas, D., Papastavrou, D., Ntatsi, G., **Ropokis, A.**, Olympios, C., Hartman, H., Schwarz, D., 2009. Interactive effects of grafting and Mn-supply on growth, yield and nutrient uptake by tomato. HortScience 44, 1978-1982.
2. Savvas, D., Savva, A., Ntatsi, G., **Ropokis, A**., Karapanos, I., Krumbein, A., Olympios, C., 2011. Effects of three commercial rootstocks on mineral nutrition, fruit yield and quality in salinised tomatoes. Journal of Plant Nutrition and Soil Science 174 (1), 154-162.
3. Petropoulos S.A., Olympios C., **Ropokis A**., Vlachou G., Ntatsi G., Paraskeuopoulos A., Passam H.C., 2014. Fruit volatile, quality and yield of watermelon as affected by grafting. Journal of Agricultural Science and Technology Journal of Agricultural Science and Technology, 16 (4), pp. 873-885.
4. Savvas, D., Oztekin G., Tepecikc, M., Ntatsi, G., **Ropokis, A**., Tüzel, Y., and Schwarzd, D., 2015. Impact of grafting and rootstock on nutrient to water uptake ratios during the vegetative stage of tomato grown hydroponically. Journal of Horticultural Science and Biotechnology, 92 (3), 294-302.
5. **Ropokis, A**., Ntatsi, G., Kittas, C., Katsoulas, N., Savvas, D. Impact of cultivar and grafting on nutrient and water uptake by sweet pepper (Capsicum annuum L.) grown hydroponically under Mediterranean climatic conditions. Front Plant Sci. 2018, 9:1244.
6. **Ropokis, A**., Ntatsi, G., Kittas, C., Katsoulas, N., Savvas, D. Effects of temperature and grafting on yield, nutrient uptake, and water use efficiency of a hydroponic sweet pepper crop. Agronomy, 9 (2), 110.
7. **Ropokis, A.,** Ntatsi, G., Rouphael, Y., Kotsiras, A., Kittas, C., Katsoulas, N., Savvas, D. Responses of pepper cultivated in a closed hydroponic system to variable Ca concentrations in the supplied nutrient solution. Journal of Plant Nutrition and Soil Science (In Press).

II. Publications in Proceedings of International Congresses and Symposia

1. **Ropokis, A.,** 2011. Design and development of innovative distance learning material based on 4MAT Model of Mc Carthy and comparison with distance learning material in EAP. An application on the grafting of herbaceous vegetables. 6th International Conference in Open & Distance Learning, Loutraki, Greece, 50-57.
2. Savvas, D., Ntatsi, D., Moiras, N., Tsakalidis, A., **Ropokis, A.**, Liopa-Tsakalidi, A. 2012. Impact of grafting and rootstock on the responses of cucumber to heavy metal stress. Acta Horticulturae, 960, pp. 49-56.

3. Savvas, D., **Ropokis, A**., Ntatsi, G., Kittas, C. 2016. Current situation of greenhouse vegetable production in Greece. Acta Horticulturae, 1142, 443-448.
4. **Ropokis, A**, Savvas, D., Giagtzoglou, P., Ginosatis, S., Ntatsi, G., Kittas, C., Katsoulas, N., 2017. Nutrient uptake concentrations of a pepper crop under Mediterranean climate conditions. Acta Horticulturae, 1170, 687-693.
5. Savvas, D., Ntatsi, G., Vlachou, M., Vrontani, C., Rizopoulou, E., Fotiadis, C., **Ropokis, A.**, Tampakaki, A., 2018. Impact of different rhizobial strains and reduced N supply on growth, yield and nutrient uptake in cowpea grown hydroponically. Acta Horticulturae, doi: 10.17660/ActaHortic.2018.1227.52
6. Savvas, D., Tsopelopoulos, K., Vourdas, C., Chatzigiakoumis, E., **Ropokis, A**., Ntatsi, G., 2019. Can grafting onto suitable rootstocks contribute to less discharge of drainage water in semi-closed soilless cultivations of tomato? XI International Symposium on Protected Cultivation in Mild Winter Climates & I International Symposium on Nettings and Screens in Horticulture, January 27-31, 2019, Tenerife (Spain - Canary Islands) Acta Horticulturae, (submitted).
7. M. Lykogianni, E. Bempelou, G. Ntatsi, I. Karavidas, **A. Ropokis**, K.A. Aliferis and D. Savvas (2020). Spinosad residues in hydroponically grown tomato cultivation. Acta Horticulturae (In Press)
8. G. Ntatsi, K.A. Aliferis, M. Lykogianni, I. Kalambokis, M. Chatzigianni, D. Yfantopoulos, **A. Ropokis** and D. Savvas (2020). Effects of cultivation system and salinity stress on yield and metabolite composition of dandelion (Taraxacum officinale). Acta Horticulturae (In Press)
9. Ntanasi, T., Ntatsi, G., Karavidas, I., Vamvakouris, N., Oikonomou, C., **Ropokis, A.,** Savvas, D., 2020. Impact of drought stress on the fruit quality of different Greek tomato landraces. Acta Horticulturae (In Press).

III. <u>Abstracts and Posters in Proceedings of Internationa Congresses and Symposia</u>

1. Savvas D., Ntatsi, G., Ropokis, A., 2013. Impact of grafting and rootstock genotype on mineral uptake by fruit vegetables. 1st Meeting of Cost Action FA 1204, 11-12 March, 2013, Athens, Greece, Book of Abstracts, pp. 61
2. Savvas, D., Oztekin, G.B., Tepecik, M., Papanikolaou, A., Katsiki, V., Ropokis, A., Ntatsi, G., 2013. Effects of grafting and rootstock genotype on nutrient uptake by tomato 2nd Meeting of Cost Action FA 1204, 11-14 November 2013, Murcia, Spain Book of Abstracts.
3. Karapanos, I., **Ropokis, A.**, Ntatsi, G., Savvas D., 2014. Impact of grafting on yield and fruit quality of pepper (Capsicum annuum l.) grown under greenhouse conditions. 1st Annual Meeting of Cost Action FA 1204, Λισαβόνα, Πορτογαλία, 20-24 Οκτωβρίου 2014, Book of Abstracts, pp. 58.
4. Sabatino, L., **Ropokis, A**., Bernabei, G., Ntatsi, G., Katsoulas, N., D'Anna, F., Savvas, D., 2016 Interactions of grafting and shading in a greenhouse pepper crop COST FA1204 Conference on Vegetable Grafting, Pula, Croatia, 19-21 September 2016, Book of Abstracts, pp. 67.
5. Savvas, D., Tsopeloupos, K., Filopoulou, S., Vourdas, C., Chatzigiakoumis, M., Panagi, P., Fanourakis, N., **Ropokis, A**., Ntatsi, G., 2017. Differences in the mode of salt tolerance between self-rooted and grafted tomato cultivars and their impact on modeling NaCl accumulation in a closed hydroponic system. VII South-Eastern Europe Symposium on Vegetables and Potatoes, Maribor, Slovenia, 20-23 June 2017, Book of Abstracts, pp. 67.
6. Ntatsi, G., Vrontani, C., Vlachou, M., Rizopoulou, E., Fotiadis, C., **Ropokis, A.**, Tampakaki, A., Savvas, D. 2017. Impact of different rhizobial strains and reduced N supply on growth and biological N2-fixation in cowpea grown hydroponically. VII South-Eastern Europe

Symposium on Vegetables and Potatoes, Maribor, Slovenia, 20-23 June 2017, Book of Abstracts, pp. 61.

7. Ntatsi, G., Vrontani, C., Vlachou, M., Rizopoulou, E., Fotiadis, C., **Ropokis, A.**, Tampakaki, A., Savvas, D. 2017. Impact of different rhizobial strains and reduced N supply on growth and biological N2-fixation in cowpea grown hydroponically. GreenSys (International Symposium on New Technologies for Environment Control, Energy-saving and Crop Production in Greenhouse and Plant Factory) Beijing, China 20-24 August 2017, Book of Abstracts, pp. 133.
8. Savvas, D., Ntatsi, G., Vrontani, C., Vlachou, M., Rizopoulou, E., Fotiadis, C., **Ropokis, A.**, Tampakaki, A., 2017. Impact of rhizobia inoculation on cowpea performance under nitrogen stress International Conference LEGATO-EUROLEGUME, Advances in grain legume cultivation and uses for a more competitive value-chain. Novi Sad, Serbia, 27-28 Σεπτεμβρίου 2017, Book of Abstracts, pp. 106.
9. Savvas, D., Tsopelopoulos, K., Vourdas, C., Chatzigiakoumis E., **Ropokis, A**., Ntatsi, G., 2019. Can grafting onto suitable rootstocks contribute to less discharge of drainage water in semi-closed soilless cultivations of tomato? XI International Symposium on Protected Cultivation in Mild Winter Climates & I International Symposium on Nettings and Screens in Horticulture, January 27-31, 2019, Tenerife (Spain - Canary Islands).
10. Ntatsi, G., Karavidas, I., Lykogianni, M., Tampakaki, A, **Ropokis, A.**, Aliferis, K., Savvas, D., 2019. Metabolic profile of cowpea plants inoculated with different rhizobia strains and subjected to drought stress conditions. Third International Legume Society Conference ILS 3, 2019, May 21-24, Poznan, Poland.

IV. Publications in Proceedings of National Congresses and Symposia

1. Ολύμπιος, Χ., Πετρόπουλος, Σ., **Ροπόκης**, **Α.,** Βλάχου, Γ., Παυλόπουλος, Σ., Παρασκευόπουλος, Α. Μελέτη της επίδρασης του υποκειμένου στην ανάπτυξη του φυτού, την παραγωγή και την ποιότητα του καρπού του καρπουζιού (*Citrullus lanatus*). Πρακτικά του 24^{ου} συνεδρίου της ελληνικής Εταιρίας της Επιστήμης των Οπωροκηπευτικών, Βέροια 2009.
2. Σάββας Δ., Ντάτση Γ., Κοντοπούλου Χ.Κ., **Ροπόκης Α.,** Κότσιρας Α., Δημόπουλος Β., Χανής Δ. 2011. Βελτιστοποίηση της διαχείρισης της άρδευσης σε καλλιέργεια αγγουριάς σε τρεις διαφορετικούς τύπους ελαφρόπετρας. Πρακτικά Ελληνικής Εταιρείας Επιστήμης Οπωροκηπευτικών, τόμος 15Α, 250-252.
3. **Α. Ροπόκης**, Ν. Κατσούλας, Κ. Κίττας, Α. Κότσιρας, Κ. Λαγού, Δ. Σάββας. Προσομοίωση συσσώρευσης Ca σε κλειστό υδροπονικό σύστημα με καλλιέργεια πιπεριάς. Πρακτικά του 8^{ου} Εθνικού συνεδρίου της ελληνικής Εταιρείας Γεωργικών Μηχανικών Ελλάδος, Βόλος 25-26 Σεπτεμβρίου 2013.
4. Σάββας, Δ., Oztekin, G.B., Tepecik, M., Παπανικολάου, Α., Κατσίκη, Β., **Ροπόκης. Α**, Ντάτση, Γ., 2013, Επίδραση του εμβολιασμού στην απορρόφηση θρεπτικών στοιχείων σε καλλιέργεια τομάτας σε κλειστό υδροπονικό σύστημα, 26ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Επιστήμης Οπωροκηπευτικών, Καλαμάτα, 15-18 Οκτωβρίου 2013, Περιλήψεις Ανακοινώσεων.
5. Σάββας, Δ., Πατεράκης, Π., Βουρναδάκη, Π., **Ροπόκης. Α.**, Ντάτση, Γ., 2013. Συγκριτική αξιολόγηση δύο τύπων ελαφρόπετρας, πετρο-βάμβακα, περλίτη και κόκου σε καλλιέργεια τομάτας. 26ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Επιστήμης Οπωροκηπευτικών, Καλαμάτα, 15-18 Οκτωβρίου 2013, Περιλήψεις Ανακοινώσεων, τόμος Β, 26-29
6. Σάββας, Δ., Τσοπελόπουλος, Κ., Φιλοπούλου, Σ., Βούρδας, Χ., Χατζηγιακουμής, Μ., Παναγή, Π., Φανουράκης, Ν., **Ροπόκης, Α.**, Ντάτση, Γ., 2018. Διαφορές στον μηχανισμό ανοχής στην αλατότητα μεταξύ αυτοφυών και εμβολιασμένων υβριδίων τομάτας και η σημασία τους για

τη μοντελοποίηση της συσσώρευσης Na σε κλειστό υδροπονικό σύστημα. 28ο Συνέδριο της Ελληνικής Εταιρείας της Επιστήμης των Οπωροκηπευτικών (ΕΕΕΟ), Θεσσαλονίκη στις 16-20 Οκτωβρίου 2017 (υποβλήθηκε για δημοσίευση).

7. **Α. Ροπόκης,** Γ. Ντάτση, Κ. Κίττας, Ν. Κατσούλας, Δ. Σάββας. Επίδραση της συγκέντρωσης ασβεστίου στο νερό άρδευσης σε καλλιέργεια πιπεριάς σε κλειστό υδροπονικό σύστημα. 28ο Συνέδριο της Ελληνικής Εταιρείας της Επιστήμης των Οπωροκηπευτικών (ΕΕΕΟ), Θεσσαλονίκη στις 16-20 Οκτωβρίου 2017 (υποβλήθηκε για δημοσίευση).
8. Ντάτση, Γ., Βροντάνη, Χ., Βλάχου, Μ, Καραβίδας, Ι., **Ροπόκης, Α**., Ταμπακάκη, Α., Σάββας, Δ., 2019. Επίδραση διαφορετικών στελεχών αζωτοδεσμευτικών βακτηρίων και μειωμένης παροχής αζώτου στην ανάπτυξη και τη βιολογική αζωτοδέσμευση υδροπονικής καλλιέργειας μαυρομάτικου φασολιού. 28^{o} Συνέδριο της Ελληνικής Εταιρείας της Επιστήμης των Οπωροκηπευτικών (ΕΕΕΟ), Θεσσαλονίκη, 16-20 Οκτωβρίου 2017 (υποβλήθηκε για δημοσίευση.
9. Γ. Ντάτση, Ι. Καραβίδας, Γ. Γιαννίκος, **Α. Ροπόκης**, Α. Ταμπακάκη, Δ. Σάββας. Επίδραση διαφορετικών στελεχών αζωτοδεσμευτικών βακτηρίων και μειωμένης παροχής νερού στην ανάπτυξη και την βιολογική αζωτοδέσμευση αμπελοφάσουλου. 28ο Συνέδριο της Ελληνικής Εταιρείας της Επιστήμης των Οπωροκηπευτικών (ΕΕΕΟ), Θεσσαλονίκη στις 16-20 Οκτωβρίου 2017 (υποβλήθηκε για δημοσίευση).

V. Abstracts of oral presentations in Proceedings of National Congresses and Symposia

1. Savvas D., Ntatsi, G., **Ropokis, A**., 2013. Impact of grafting and rootstock genotype on mineral uptake by fruit vegetables. 1st Meeting of Cost Action FA 1204, 11-12 March, 2013, Athens, Greece, Book of Abstracts, pp. 61.
2. Savvas, D., Oztekin, G.B., Tepecik, M., Papanikolaou, A., Katsiki, V., **Ropokis, A**., Ntatsi, G., 2013. Effects of grafting and rootstock genotype on nutrient uptake by tomato 2nd Meeting of Cost Action FA 1204, 11-14 November, 2013, Murcia, Spain Book of Abstracts.

VI. Articles in technical - scientific journals

1. Σάββας, Δ., Πατεράκης, Π., Βουρναδάκη, Π., **Ροπόκης, Α.**, Ντάτση, Γ., 2014. Αξιολόγηση υποστρωμάτων σε υδροπονική καλλιέργεια τομάτας. Γεωργία - Κτηνοτροφία, 1/2014, σελ. 64-70.