

Evaluation of rapid analysis systems for on-farm nitrate analysis in vegetable cropping

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Abstract

Small analytical systems enabling rapid on-farm analysis may be useful for monitoring crop and soil nutrient status. Two commercially available, rapid analysis systems were evaluated for analysis of $[\text{NO}_3^-]$ in aqueous standard solutions (AS), and in soil solution (SS), plant sap (PS) and fertigation nutrient solutions (NS) obtained from tomato crops. The systems were the Merck RQFlex reflectometer and the Hanna Instruments C115 Grow Master colorimeter. $[\text{NO}_3^-]$ in AS covered the analytical range of each instrument, being 0.3-3.2 and 0.3-16.1 mmol L⁻¹, respectively, for the reflectometer and colorimeter. $[\text{NO}_3^-]$ were 5-14 mmol L⁻¹ for SS, 2.3-33 mmol L⁻¹ for NS, and 63-158 mmol L⁻¹ for PS. Accuracy was evaluated using linear regression against (i) known concentration for AS, and (ii) laboratory analysis for SS, PS and NS. For the reflectometer with AS, SS and PS, the linear regression had slopes of 1 ± 0.08 and intercepts of < 0.6 mmol L⁻¹. In NS up to 9 mmol L⁻¹, there was a similar slope, with an intercept of 1.3 mmol L⁻¹. In the three most concentrated NS samples, there was a larger relative error that was attributed to faulty dilution. Coefficients of determination were always $r^2 \geq 0.94$. The reflectometer was generally accurate for all media examined; it was rapid (1 min per analysis) and simple to use. The colorimeter was generally similarly accurate; however, it was slower, required reagent preparation and was subject to colour interferences. This work demonstrated that the reflectometer can provide rapid and accurate on-farm measurement of $[\text{NO}_3^-]$ in SS, PS and NS.

Additional key words: nutrient management, nutrient solution, plant sap, quick-test, soil solution, tomato.

Resumen

Evaluación de sistemas rápidos para análisis en campo de nitrato en cultivos hortícolas

Los sistemas rápidos que permiten análisis en campo pueden ser útiles para monitorizar el estado nutricional del cultivo y del suelo. Se evaluaron dos sistemas analíticos rápidos para analizar $[\text{NO}_3^-]$ en soluciones acuosas (AS), solución del suelo (SS), muestras de savia (PS) y soluciones de fertirriego (NS). Se evaluó el reflectómetro RQFlex de Merck y el colorímetro C115 de Hanna. La $[\text{NO}_3^-]$ en AS cubrió el rango analítico de cada instrumento y fue 0.3-3.2 y 0.3-16.1 mmol L⁻¹, respectivamente, para el reflectómetro y el colorímetro. La $[\text{NO}_3^-]$ alcanzó 5-14 mmol L⁻¹ para muestras de SS, 2.3-33 mmol L⁻¹ para NS, y 63-158 mmol L⁻¹ para PS. Para valorar la exactitud de los equipos se usaron regresiones lineales. Para el reflectómetro con muestras de AS, SS y PS, la regresión lineal tuvo pendientes de 1 ± 0.08 e interceptos de < 0.6 mmol L⁻¹. En NS para valores de hasta 9 mmol L⁻¹, la pendiente fue similar entre equipos, con un intercepto de 1.3 mmol L⁻¹. En las NS más concentradas, hubo un error relativamente grande que se atribuye a fallos en la dilución. Los r^2 fueron siempre ≥ 0.94 . El reflectómetro fue generalmente preciso en los medios evaluados; fue rápido (1 min/análisis) y simple de usar. El colorímetro tuvo generalmente una exactitud similar; sin embargo, las medidas fueron más lentas, requiere preparación de reactivos y presentó interferencias con el color de las muestras. Este trabajo demuestra que el reflectómetro permite una medida rápida y precisa en campo de la $[\text{NO}_3^-]$ en muestras de SS, PS y NS.

Palabras clave adicionales: análisis rápido, manejo de nutrientes, savia, solución nutritiva, solución de suelo, tomate.

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Introduction

Intensive vegetable production systems are generally characterised by high inputs of nitrogen (N) and low recovery of applied N by vegetable crops (Pratt, 1984; Balusubramanian *et al.*, 2004). Appreciable nitrate leaching losses are commonly measured from these systems (e.g. Pratt, 1984; Ramos *et al.*, 2002; Vázquez *et al.*, 2006; Thompson *et al.*, 2007a). Along much of the Mediterranean Coast of Spain, intensive vegetable production systems are associated with appreciable nitrate contamination of aquifers. In accordance with the EU Nitrate Directive and Water Framework (OJ, 1991, 2000), these regions are required to implement management practices that will reduce this contamination.

The combined use of (i) drip irrigation, (ii) fertigation systems and (iii) programmable controllers is increasing in intensive vegetable production systems. Such combined systems are commonly used in substrate-based production (Adams, 2002), and are being increasingly adopted in soil-based vegetable production such as in the greenhouse-based vegetable production system of south-eastern Spain (Carreño and Magan, 1999; Pérez-Parra and Céspedes, 2001). These combined systems enable frequent application of precise volumes of water with specified concentrations of selected nutrients, providing vegetable growers with the technical capacity to “spoon-feed” nutrients to crops. Therefore these combined systems have the potential for a high level of N management. However, where these combined systems are available, growers’ management is generally based on fixed programs developed from local experience (Thompson *et al.*, 2007b). To take advantage of the technical capacity of combined drip irrigation/fertigation systems for precise N application, the adoption of improved N management practices is required.

Prescriptive-corrective management has been proposed as an optimal approach to crop N management (Giller *et al.*, 2004). Prescriptive management refers to nutrient management programs prepared prior to planting that anticipate crop nutrient requirements (Giller *et al.*, 2004). Corrective management refers to the use of monitoring procedures during cropping that enable adjustment of nutrient management practices to correct detected deficiencies or excesses (Giller *et al.*, 2004). Because of small size of fields/greenhouse and the overall intensity of management, intensive vegetable production systems, with advanced drip irrigation/fertigation systems are well-suited to the introduction of

combined prescriptive-corrective nutrient management systems.

Regular on-farm monitoring of crop and soil nutrient status with rapid analysis systems is a promising approach for corrective management. Analysis of NO_3^- concentration in soil solution obtained with ceramic cup suction samplers from the root zone (Hartz *et al.*, 1993; Burt *et al.*, 1995) and in petiole sap (Hartz *et al.*, 1994; Hochmuth, 1994, 2007; Olsen and Lyons, 1994) have been suggested as methods for on-going monitoring of, respectively, the immediately available soil N and crop N status. Recent studies within greenhouse vegetable production systems (Granados *et al.*, 2005, 2007; Gallardo *et al.*, 2006) have demonstrated that soil solution nitrate concentration is very sensitive to crop N management. Compared to conventional soil sampling with its associated extraction procedures, soil solution sampling with ceramic cup suction samplers is much simpler and more suited for regular, on-farm monitoring. In a pepper crop, Granados *et al.* (2007) used prescriptive-corrective management, based on modelling crop N uptake for the prescriptive component, and monitoring and control of root zone soil solution NO_3^- concentration for the corrective component, to appreciably reduce N application and NO_3^- leaching loss. An appreciable and increasing number of commercial farmers in the greenhouse-based vegetable production system of Almería, Spain, are routinely using samples of soil solution, obtained with ceramic cup samplers, for the measurement and management of root zone salinity.

Samples of soil solution and plant sap can be easily and rapidly obtained within a commercial vegetable crop; the former with ceramic cup soil solution samplers, the latter using a domestic manual press. Additionally, occasional monitoring of NO_3^- concentrations of applied nutrient solutions prepared by automatic fertigation systems would ensure more precise control than currently occurs through control of pH and electrical conductivity (EC).

On-farm analysis of NO_3^- concentration in sap and soil solution samples, for crop monitoring purposes, may be preferable to laboratory analysis for reasons of immediate availability of results, cost, and the practical inconvenience of packaging and transporting samples. Small, robust and simple to use, rapid analytical systems are available for on-farm analysis of NO_3^- , which can be readily used by technical advisers or farmers without specific training in analytical chemistry (Hartz *et al.*, 1994; Burt *et al.*, 1995). Some systems based on colorimetric analysis can also analyse ammonium

(NH_4^+), potassium (K) and phosphorus (P), enabling them to assist with management of all macro-nutrients in intensive vegetable production systems. Some systems are based on ion selective electrodes, and are limited to only one nutrient.

There are reports of studies examining the use of rapid analysis systems for NO_3^- analysis. A number have been conducted with ion selective electrodes specific to NO_3^- (e.g. Hartz *et al.*, 1993, 1994; Rosen *et al.*, 1996). The media examined have been mostly soil extracts (Jemison and Fox, 1988; Hartz, 1994; Schmidhalter, 2005) or plant sap (Hartz *et al.*, 1993; Hochmuth, 1994; Rosen *et al.*, 1996). Very few studies have examined NO_3^- in the soil solution, none have done so using colorimetric approaches, and no studies have used colorimetric approaches to examine NO_3^- in the three media of most interest to intensive vegetable production, namely soil solution, plant sap, and applied nutrient solution.

This research was conducted with the primary objective of examining the accuracy of two commercially-available, rapid analysis systems, based on colorimetry, for the analysis of NO_3^- in soil solution, plant sap and complete nutrient solutions obtained from an intensive vegetable production system. Both analytical systems can also be used for analysis of NH_4^+ , K and P. Additional objectives were to assess the linearity and repeatability of the analyses, and the general practical suitability of the two analytical systems for on-farm application.

Material and methods

Two portable rapid analytical systems were evaluated for their accuracy in the analysis of NO_3^- in aqueous standard solutions, soil solution obtained with ceramic cup suction samplers, plant sap and fertigation nutrient solutions. Analytical accuracy was evaluated by comparison with (i) known concentration or (ii) laboratory analysis using an established laboratory method. The samples of soil solution, plant sap and nutrient solution were obtained from (a) greenhouses with tomato crops, grown under conditions very similar to commercial production, at the research station of the Cajamar Foundation, in El Ejido, Almería province, Spain, and (b) a commercial greenhouse located in La Cañada in Almería province in Spain. The greenhouse-based vegetable production system in which the work was conducted has been described by Castilla and Hernández (2005) and Thompson *et al.* (2007b).

All measurements using the rapid analysis systems were conducted in a laboratory in the University of Almería, in Almería, Spain. Room temperature was approximately 20°C. Samples which had been previously refrigerated were allowed to reach room temperature prior to analysis. All results are reported in units of $\text{mmol NO}_3^- \text{ L}^{-1}$. The measurements with the rapid analysis systems were conducted by a university student, with some but limited laboratory experience, which is the likely to be similar to the background of many potential users, most of whom would be technical advisers or farmers.

Systems of rapid analysis evaluated

The two rapid analysis systems examined were a reflectometer (model RQFlex, Merck, Darmstadt, Germany) and a colorimeter (model C115 Grow Master, Hanna Instruments, Rhode Island, USA). The reflectometer which measures the colour intensity of Reflectoquant® test strips (Merck, Darmstadt, Germany) is based on a colorimetric method. For NO_3^- analysis, the test strip is placed directly in the test solution for 2 s, and after a total reaction time of 1 min, the amount of light reflected from the test strip is measured, which is converted to concentration by a standard calibration previously introduced into the equipment through a bar coded plastic strip. Measured concentrations are given as $\text{mg NO}_3^- \text{ L}^{-1}$. The analytical range of the reflectometer for NO_3^- analysis, as stated by the manufacturer, is 0.1 to 3.6 mmol L^{-1} (5-225 $\text{mg NO}_3^- \text{ L}^{-1}$). The reflectometer is an analytical system developed for in-situ analysis of natural surface waters. In addition to NH_4^+ , PO_4^{3-} and K, it can be used for a wide range of analyses which are described in the web page of the manufacturer. Calibrations for five different analyses can be stored in the instrument's memory at one time.

The C115 colorimeter measures colour development as absorption at 555 nm of a solution placed within a measuring cell. For NO_3^- , a specific volume of solution is mixed with a packet of purchased reagent for 1 min, and after 6 more minutes, the absorption is measured which is converted to concentration using a calibration permanently stored in the apparatus. Measured concentrations are given as $\text{mg NO}_3^- \text{ N L}^{-1}$. The analytical range of the colorimeter for NO_3^- analysis, as stated by the manufacturer, is 0-21 mmol L^{-1} (0-300 $\text{mg NO}_3^- \text{ N L}^{-1}$). The analysis involves reduction of NO_3^- to nitrite (NO_2^-) with cadmium and the subsequent reaction of

NO_2^- with sulfanilic acid; the reagent package contains a mixture of cadmium powder, copper sulphate and sulfanilic acid.

The C115 colorimeter was developed, to be used in horticulture, for the analysis of macro-nutrients in nutrient solutions. The colorimeter has a dial to select one of three calibrations of “low”, “medium” or “high”, corresponding to concentration ranges of, respectively, 0-2, 0-11, 0-21 $\text{mmol NO}_3^- \text{N L}^{-1}$. In the work with aqueous solutions, a very similar degree of accuracy and linearity was obtained when using either: (a) the three calibrations for concentrations that corresponded to 0-2, 2-11, 11-21 $\text{mmol NO}_3^- \text{N L}^{-1}$, or when using (b) the “high” calibration for all samples across the full operating range of the colorimeter. Consequently, the subsequent analyses for nutrient solutions, plant sap and soil solution were conducted using the “high” calibration.

When NO_3^- concentrations exceeded the analytical range of an instrument, dilutions with demineralised water were made using a hand-held, laboratory pipetter and volumetric flasks. Dilutions were always necessary with plant sap, for both instruments. For nutrient solution and soil solution samples, dilution was commonly required using the RQFlex reflectometer, and required in a small number of cases with the colorimeter. Dilutions were conducted so that the NO_3^- concentration of the diluted sample was approximately mid-way within the analytical range.

Laboratory analysis of nitrate

All samples of nutrient solution, plant sap and soil solution were analysed for NO_3^- in a laboratory using the ultraviolet method (MAPA, 1994) with a bench-top spectrophotometer (model Helios a, Unicam, Leeds, UK), which is hereafter referred to as the laboratory analysis.

Accuracy and linearity of analysis of aqueous standard solutions

Aqueous standard solutions of known concentration were prepared, using demineralised water, to cover the NO_3^- measurement range for each of the analytical systems. For the reflectometer, standard solutions of 0.3, 0.8, 1.6, 2.4 and 3.2 $\text{mmol NO}_3^- \text{L}^{-1}$ were prepared. For the colorimeter, the five solutions used with the reflectometer and five additional standard solutions of 6.5,

9.7, 12.9, 16.1 and 19.3 $\text{mmol NO}_3^- \text{L}^{-1}$ were examined. Aqueous standard solutions with NO_3^- concentrations of $\leq 16.1 \text{ mmol NO}_3^- \text{L}^{-1}$ were prepared from a purchased 16.1 $\text{mmol NO}_3^- \text{L}^{-1}$ (1 g $\text{NO}_3^- \text{L}^{-1}$ as NaNO_3) standard solution (Merck, Darmstadt, Germany). The 19.3 $\text{mmol NO}_3^- \text{L}^{-1}$ standard was prepared from a 10 g $\text{NO}_3^- \text{L}^{-1}$ (161 mmol L^{-1}) stock solution. As the standards examined covered the analytical range of each instrument, these analyses also enabled an assessment of the linearity of the analyses.

Accuracy of analysis of soil solution

Eleven samples of root zone soil solution, with a range of 5-19 $\text{mmol NO}_3^- \text{L}^{-1}$ were obtained with individual ceramic cup suction samplers (Model 1900, Soil Moisture Co., Santa Barbara, CA, USA) installed at 15 cm depth, 10 cm to the side of tomato plants. Samples were collected after applying a vacuum of -65 kPa for 24 h, and were stored at 3°C immediately after collection. Samples were obtained from a greenhouse at the research station of the Cajamar Foundation and from a commercial greenhouse. The compositions of two representative soil solution samples are presented in Table 1.

Accuracy of analysis of plant sap

Twelve samples of plant sap, with NO_3^- concentrations of 41-158 mmol L^{-1} , were analysed. Sap was obtained from crushed petioles of fully expanded leaves of mature tomato plants grown in greenhouses. Each sap sample was obtained from nine leaves taken from the same relative position on different plants, selected at random, which had received identical management. Samples were obtained from different tomato crops at the research station of the Cajamar Foundation, and, at intervals, from a tomato (*Solanum lycopersicum* L.) crop in a commercial greenhouse. All samples were collected on a sunny day between 10:00 to 13:00 hours. Leaves were stored in sealed plastic bags and transported to the laboratory. In the laboratory, petioles were separated from blades, cleaned with distilled water and cut in 1-cm pieces that were then pressed with a domestic manual press. Samples analysed with the colorimeter were filtered and treated with activated carbon prior to analysis to clarify the samples in order to reduce interferences with colour measurement.

Table 1. Chemical composition of representative individual samples of soil solution and nutrient solution analysed in this study. Data are presented for electrical conductivity (EC) (dS m⁻¹), and for concentrations (mmol L⁻¹) of NO₃⁻, sulphate (SO₄²⁻), bicarbonate (HCO₃⁻), phosphorus (P), chloride (Cl⁻), potassium (K⁺), calcium (Ca²⁺) and magnesium (Mg²⁺). All analyses were conducted using methods described in MAPA (1994)

Type of solution	EC	[NO ₃ ⁻]	[SO ₄ ²⁻]	[HCO ₃ ⁻]	[P]	[Cl ⁻]	[K ⁺]	[Ca ²⁺]	[Mg ²⁺]
Soil solution	3.5	9.6	9.8	9.6	0.2	1.1	13.8	6.6	3.7
Soil solution	2.9	12.4	5.2	3.4	1.6	3.1	11.0	3.9	2.7
Nutrient solution	2.6	9.0	5.5	0.5	1.2	0.7	11.3	3.6	3.5
Nutrient solution	2.7	24.2	1.6	n.a.	1.2	0.9	5.3	6.5	3.0

n.a.: not analysed

Accuracy of analysis of fertigation nutrient solutions

Nine complete fertigation nutrient solutions collected from irrigation drippers and from a concentrated fertigation “stock” solution, with NO₃⁻ concentrations of 2.3, 2.9, 3.2, 5.5, 6.7, 9.0, 12.2, 24.2, and 33.0 mmol L⁻¹, were used to assess the accuracy of analysis in nutrient solutions. After collection, samples were maintained at 3°C until analysis. Three replicate analyses were conducted for each sample with each rapid analysis system and with the established laboratory method. Mean data were used to assess accuracy. The precision of the two rapid analysis systems and the established laboratory method was assessed by calculating the average coefficient of variation of these measurements for each analytical system. The composition of two representative nutrient solution samples are presented in Table 1.

Statistical analyses

The accuracy of the measurements with the rapid analysis systems were assessed by linear regression analysis against known concentrations for AS and laboratory analysis for SS, PS and NS, for each data set (i.e. each combination of individual rapid analysis system and media). For each combination of instrument and sample type, the fitted regression line was compared with the 1:1 line for statistically significant differences ($P < 0.05$) with respect to slope and intercept. All statistical analyses were conducted using the Statgraphics Plus ver. 4.1. (Manugistic Co. Rockville, Maryland, USA). Unless stated otherwise, statistical analyses were significant at $P < 0.05$.

Results

Aqueous standard solutions

With aqueous standard NO₃⁻ solutions, the reflectometer provided excellent agreement with the known values. There was a linear response across the concentration range of 0.3 to 3.2 mmol L⁻¹ (Fig. 1a, Table 2), this range is very similar to the stated operating range of 0.1 to 3.6 mmol L⁻¹.

The colorimeter provided very good agreement with known values, and a linear response across the concentration range of samples analysed of 0.3 to 16.1 mmol L⁻¹, using either the three calibrations for specific concentration ranges, or using the “high” calibration for all samples (Figs. 1b and 1c; Table 3). The colorimeter gave an “out of range” signal with the 19.3 mmol L⁻¹ sample, indicating that it was unable to measure in the upper part of the range stated by the manufacturer of 0 to 21 mmol L⁻¹. With both calibration systems, the linear regressions analyses against known concentrations gave equations with slopes of 1 ± 0.07 , intercepts of 0.05-0.18 mmol L⁻¹, and coefficient of determination (r^2) values of 0.982-0.990 (Figs 1b and 1c; Table 3).

There were no statistically significant differences ($P < 0.05$) between the slopes and intercepts of the regression analyses and the 1:1 lines for the reflectometer (Table 2), and for the colorimeter using either of the two calibration approaches (Table 3). There were no statistically significant differences in slope or intercept between the linear equations for both calibration approaches used with the colorimeter.

Soil solution

In soil solution, the reflectometer provided very similar results to the laboratory analysis across the

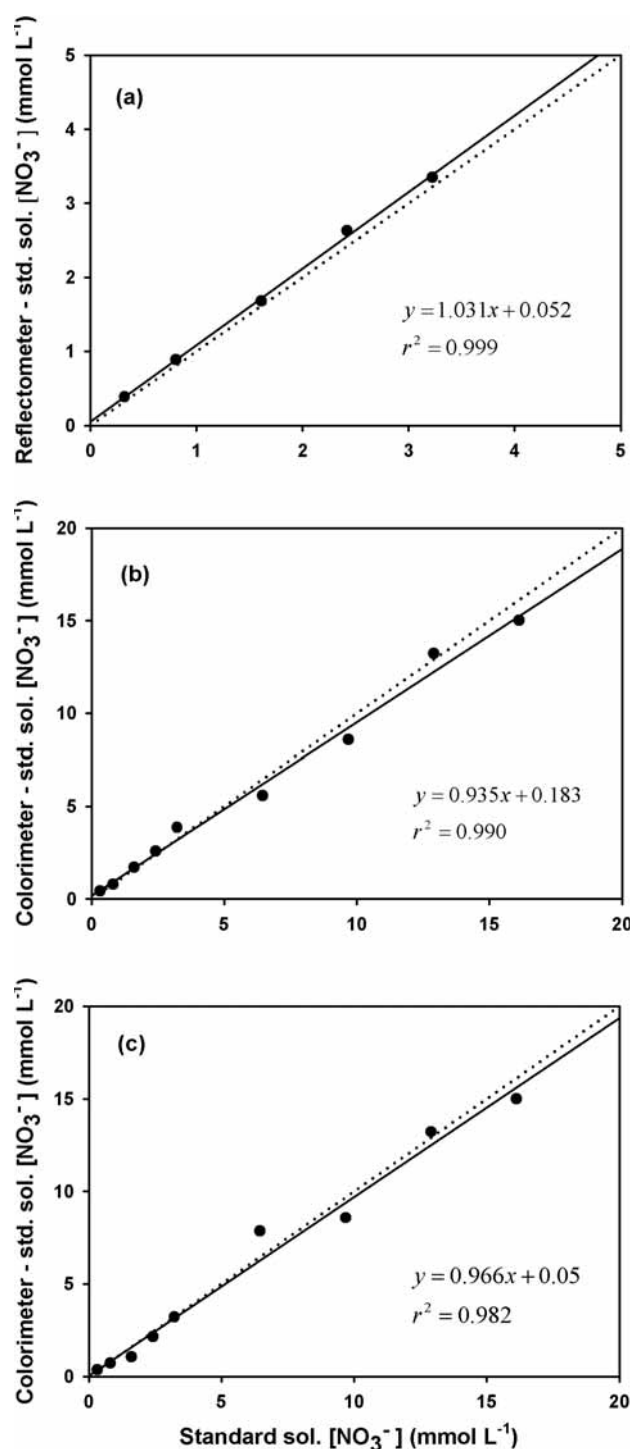


Figure 1. Plot of measurement of nitrate concentration measured with rapid analytical systems against known concentration in aqueous standard solutions, as measured with (a) the reflectometer, (b) the colorimeter using three different calibrations for different ranges, and (c) the colorimeter using the “High” calibration for the entire concentration range. The broken line is the theoretical 1:1 line.

concentration range examined of 5–19 mmol L⁻¹ (Fig 2a; Table 2). The colorimeter provided generally good agreement with the laboratory analysis but with notable dispersion of data (Fig. 2b; Table 3). The degree of dispersion was reflected in the relative difference of the slope and intercept values from those of the 1:1 line (Table 3), and in the r^2 value which was the lowest obtained for an individual data set in the present study (Tables 2 and 3). Comparison with the 1:1 lines showed no statistically significant differences in slope or intercept for either instrument (Tables 2 and 3).

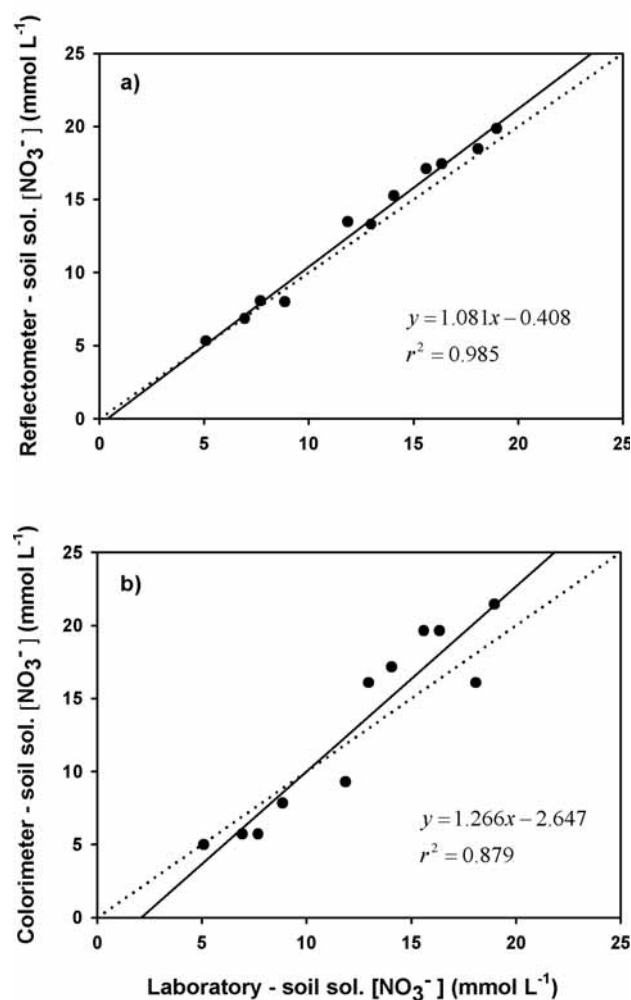


Figure 2. Plot of measurement of nitrate concentration measured with rapid analytical system against laboratory analysis in soil solution, as measured with (a) the reflectometer, and (b) the colorimeter. The broken line is the theoretical 1:1 line.

Table 2. Results of linear regression analysis comparing analyses for nitrate concentration made with the reflectometer to known concentrations of standard aqueous solutions, and laboratory analysis for soil solution, plant sap, and fertigation nutrient solutions. Presented are the equations, coefficients of determination (r^2), and results of the comparison of each equation with the corresponding 1:1 line. Symbols * and ** represent statistically significant differences between lines at $P < 0.05$ and 0.01, respectively, and n.s. indicates no statistically significant differences

Media	Concentration range (mmol L ⁻¹)	Equation, coefficient of determination (r^2)	Comparison with 1:1 line	
			Slope	Intercept
Standard solutions	0.3 to 3.2	$y = 1.031x + 0.052$, $r^2 = 0.999$	n.s.	n.s.
Soil solution	5.1 to 19.0	$y = 1.081x - 0.408$, $r^2 = 0.985$	n.s.	n.s.
Plant sap	55 to 158	$y = 0.949x + 0.526$, $r^2 = 0.942$	n.s.	n.s.
Nutrient solution	2.3 to 33	$y = 1.193x + 0.172$, $r^2 = 0.997$	*	**
Nutrient solution	2.3 to 9.0	$y = 0.935x + 1.295$, $r^2 = 0.976$	n.s.	*

Plant sap

In the plant sap samples, both the reflectometer and colorimeter provided good agreement with the laboratory analysis across the full range of NO_3^- concentrations examined of 55-158 mmol L⁻¹ (Fig. 3a and 3b; Tables 2 and 3). For the linear regressions for both instruments, there were no statistically significant differences in slope or intercept from the 1:1 lines (Tables 2 and 3).

Nutrient solutions

In the nutrient solutions, the reflectometer provided very good agreement with the laboratory analysis up to

9.0 mmol NO_3^- L⁻¹ (Fig. 4a; Table 2). Overestimates of 19-28% for the three higher concentrations (12.2-33.0 mmol L⁻¹) caused the linear regression for the complete range to overestimate by 19% (Fig 4a; Table 2). The accuracy at concentrations of ≤ 9 mmol NO_3^- L⁻¹ and the systematic error at higher concentrations, in which dilution was required, suggest that error with higher concentration samples was most probably due to faulty dilution. For the complete range, there were statistically significant differences ($P < 0.05$) between the derived linear regression and the 1:1 line with respect to slope and intercept (Table 2). For the 6 samples to 9.0 mmol L⁻¹, there were no statistically significant differences in

Table 3. Results of linear regression analysis comparing analyses for nitrate concentration made with the colorimeter to known concentrations of standard aqueous solutions, and laboratory analysis for soil solution, plant sap, and fertigation nutrient solutions. Presented are the equations, coefficients of determination (r^2), and results of the comparison of each equation with the corresponding 1:1 line. Symbols * and ** represent statistically significant differences between lines at $P < 0.05$ and 0.01, respectively, and n.s. indicates no statistically significant differences

Media	Concentration range (mmol L ⁻¹)	Equation, coefficient of determination (r^2)	Comparison with 1:1 line	
			Slope	Intercept
Standard solutions ¹	0.3 to 16.1	$y = 0.935x + 0.183$, $r^2 = 0.990$	n.s.	n.s.
Standard solutions ²	0.3 to 16.1	$y = 0.966x + 0.050$, $r^2 = 0.982$	n.s.	n.s.
Soil solution	5.1 to 19.0	$y = 1.266x - 2.647$, $r^2 = 0.879$	n.s.	n.s.
Plant sap	55 to 158	$y = 1.011x + 6.036$, $r^2 = 0.945$	n.s.	n.s.
Nutrient solution	2.3 to 33	$y = 1.067x + 0.467$, $r^2 = 0.937$	n.s.	n.s.
Nutrient solution	2.3 to 12.2	$y = 0.942x + 1.467$, $r^2 = 0.962$	n.s.	n.s.

¹ Analysed selecting appropriate calibration from the three available calibrations of "Low", "Medium" and "High", which were respectively used for the ranges of 0-2, 2-11, and 11-21 mmol L⁻¹. ² Analysed using the "High" calibration for the entire range of concentrations examined

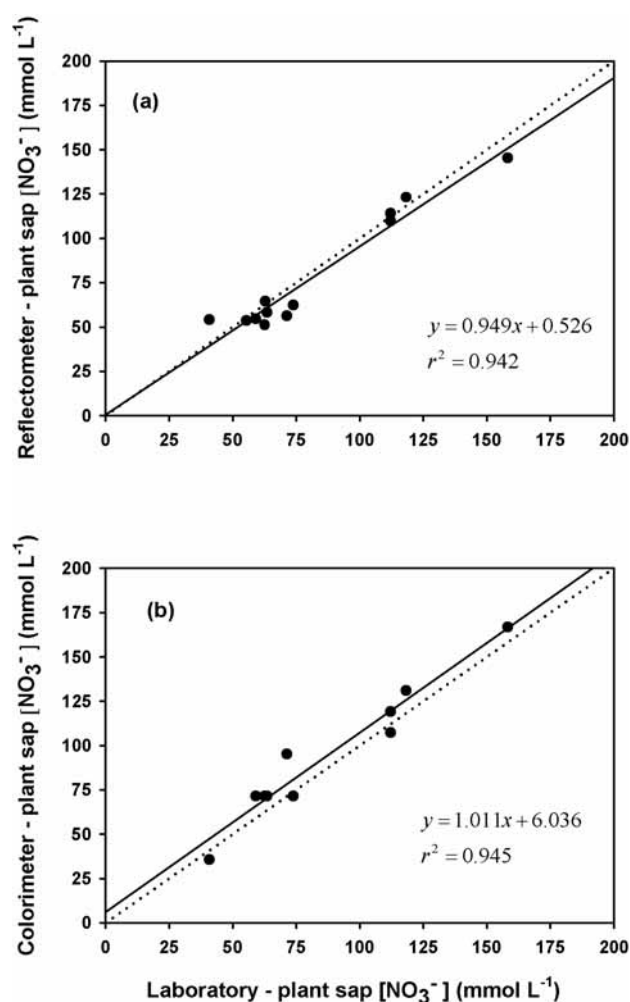


Figure 3. Plot of measurement of nitrate concentration measured with rapid analytical system against laboratory analysis in plant sap, as measured with (a) the reflectometer, and (b) the colorimeter. The broken line is the theoretical 1:1 line.

slope between the derived linear regression line and the 1:1 line. However, there was a statistically significant difference in intercept on account of the intercept value of 1.2 mmol L⁻¹ (Table 2).

There was good agreement between the colorimeter and the laboratory analyses up to 12 mmol L⁻¹ (Fig. 4b; Table 3). The two higher concentration samples of 24 and 33 mmol L⁻¹, which required dilution, had measurement errors of -18 and 23%, respectively. Inclusion of these analyses in the regression analysis for the full range reduced the coefficient of determination from 0.962 to 0.937 (Table 3). For the concentration range to 12 mmol L⁻¹ and the full concentration range, there were no statistically significant differ-

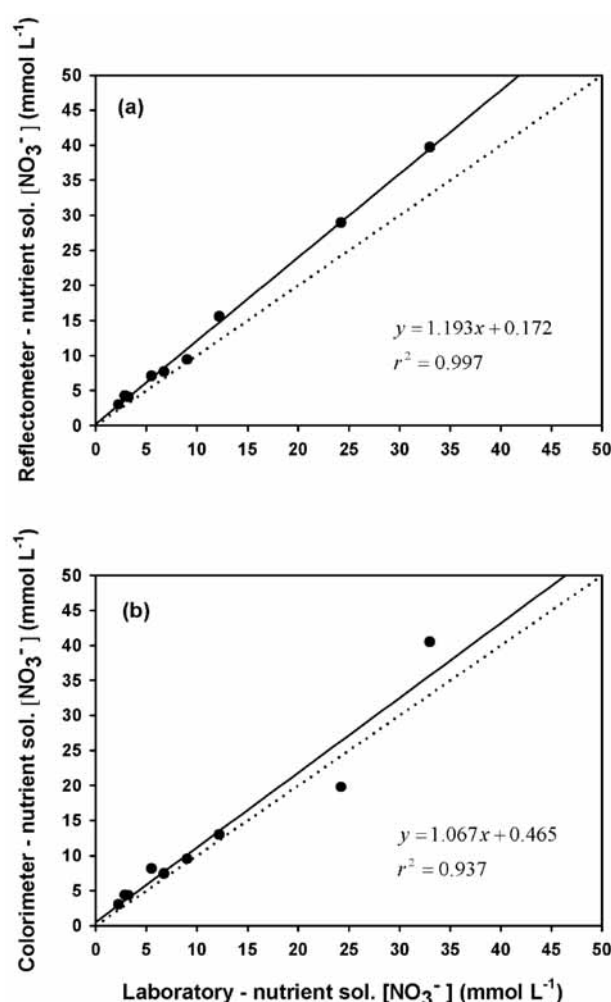


Figure 4. Plot of measurement of nitrate concentration measured with rapid analytical systems against laboratory analysis in complete nutrient solution, as measured with (a) the reflectometer, and (b) the colorimeter. The broken line is the theoretical 1:1 line.

ences between the derived linear regression and the 1:1 line (Table 3).

Precision of analysis

Precision of analysis was evaluated by calculating coefficient of variation (CV) values from the three replicate analyses of each of the nutrient solution samples. Average CV values for the reflectometer, colorimeter and the laboratory analyses were, respectively, 5.5, 11.8 and 0.95% (Table 4). These data demonstrate that, in this work, the rapid analysis systems were less precise than the laboratory analysis,

with the colorimeter being less precise than the reflectometer.

Discussion

For all solutions analysed, except for most concentrated nutrient solutions, the Merck RQFlex reflectometer provided accurate NO_3^- analysis. Excluding the most concentrated nutrient solutions, the linear regression equations, describing the relationship of reflectometer analysis to known values or laboratory analysis, had slopes of 1 ± 0.08 and intercepts of $<1.3 \text{ mmol L}^{-1}$; in three of four cases, the intercepts were $<0.6 \text{ mmol L}^{-1}$. For all type of samples, for the complete ranges examined, coefficients of determination were always $r^2 \geq 0.94$, indicating good agreement with the linear model. The overestimation with nutrient solutions with NO_3^- concentrations of $12\text{--}33 \text{ mmol L}^{-1}$ occurred where appreciable dilution was required. These dilutions and analyses were conducted early in the study by an initially inexperienced student. Samples of soil solution and plant sap which were diluted and analysed, later in the study, did not show this discrepancy. The discrepancies with the three most concentrated samples of nutrient solution were attributed to faulty dilution.

For the Hanna Instruments C115 colorimeter with aqueous standard solutions, complete nutrient solutions and plant sap, slopes of the linear regression with laboratory analysis were within 1 ± 0.07 , intercepts were close to zero, and coefficients of determination were $r^2 \geq 0.94$. In soil solution, the colorimeter was appreciably less accurate. Interference of sample colour with colour development is the most likely explanation. Soil solution samples were not clarified prior to analysis with the colorimeter as were plant sap samples for which there was comparatively much smaller dispersion from the 1:1 line.

Generally, both rapid analysis systems provided accurate analysis of NO_3^- in nutrient solutions, soil solution and plant sap. The few exceptions were attributed to

faulty dilution of the most concentrated nutrient solution samples or to interference in colour development of coloured soil solution samples. These results demonstrate how critical the process of dilution is for accurate analysis with these systems, particularly the reflectometer, which in its current form has a limited analytical range for NO_3^- . For on-farm use, it is essential that users have appropriate equipment and water for conducting dilution, and are well trained in dilution procedure. Interferences with colour development were not an issue with the reflectometer in the present work. However, it appears with the colorimeter that coloured samples require clarification. The presence of appreciable concentrations of other ions (Table 1) had no apparent effect on the accuracy of analysis with either system.

The results obtained with the reflectometer, in the present study, are generally consistent with relevant published work; there are very few relevant published studies with small portable colorimeters. The accuracy of the reflectometer for NO_3^- analysis in plant sap in the present work was similar to that reported by Jemison and Fox (1988) using a different reflectometer system. Jemison and Fox (1988) and Schmidhalter (2005) obtained good agreement between reflectometer and laboratory analysis for measurement of NO_3^- in soil extracts, with the presence of high background concentrations of extractant salts. The results of the present study are consistent with these studies in that there do not appear to be chemical interferences with NO_3^- analysis using the reflectometer for soil and plant analysis applications.

The two rapid analytical systems assessed in this work demonstrated a high level of accuracy for the analysis of NO_3^- in the various media examined. The higher CV values, obtained with both rapid analysis systems compared to laboratory analysis of nutrient solutions, indicated a lower degree of precision than for laboratory analysis. Jemison and Fox (1988) reported CV values of 1.6 to 3.5% with a reflectometer for NO_3^- analysis of aqueous solutions, suggesting that with very good technique, a relatively high level of precision can be obtained with analytical systems of this type.

Considering the present work and relevant published studies, the reflectometer has been used for accurate analysis of NO_3^- in aqueous solutions, fertigation nutrient solutions, plant sap, soil solution and soil extraction solutions. The reflectometer is relatively cheap, simple to use, requires very little operator training, performs NO_3^- analysis rapidly (1 min per analysis) and requires no sample preparation. With coloured samples such as

Table 4. Average and range of coefficients of variations (CV) expressed as % for the measurement of NO_3^- in the nutrient solutions for each method of analysis

	Laboratory	Reflectometer	Colorimeter
Average CV	0.95	5.5	11.8
Range of CV	0.26-1.8	1.2-20.9	3.2-30.0

plant sap and soil solution, no colour clarification was required. The system is very small, measuring 19 x 8 x 4 cm, and consequently is very portable. A disadvantage of the reflectometer, for horticultural applications, is the narrow analytical range for NO_3^- , which requires that most samples be diluted. This reflects that the reflectometer was developed for natural surface waters. Increasing its analytical range would considerably improve its suitability for use in horticulture. Rodrigues *et al.* (2002) reported, for the same reflectometer used in the present study, that at temperatures of $<21^\circ\text{C}$ there was a slightly negative relationship between measured NO_3^- concentration and temperature. It is suggested that samples be at $>15^\circ\text{C}$ for analysis; at lower sample temperatures, a small correction may be required to optimise accuracy (Rodrigues *et al.*, 2002). In its present form, the requirement for dilution increases the time for each analysis and provides a possible source of inaccuracy. As a general conclusion, with good analytical procedures (accurate dilution, consistent operation of equipment, cleanliness etc.) the reflectometer can provide accurate analysis of NO_3^- in horticultural applications, with a relatively high degree of precision.

Compared to the reflectometer, the colorimeter had similar overall accuracy and a larger analytical range. However, analysis was appreciably slower (7–8 min per analysis), and it was necessary to prepare reagents. Clarification appears to be a requirement for coloured samples (sap, soil solution) which increases the amount of sample handling and the time required by approximately 30 min.

Of the two instruments evaluated in this study, the reflectometer is recommended for NO_3^- analysis for horticultural applications on account of the rapidity and simplicity of analysis, ease of use, accuracy, good precision, and capacity to analyse coloured samples. Good results for the rapid analysis of NO_3^- in plant sap and soil solution have also been obtained with small nitrate-selective electrode systems (Hartz *et al.*, 1994; Rosen *et al.*, 1996). Ion selective electrode rapid analysis systems require a specific instrument for each ion being measured, and currently there is only a very limited range of such ion specific instruments that are commercially available. The reflectometer is extremely versatile, with one instrument being able to analyse many different parameters, and being able to retain five different calibrations within its memory.

In spite of its currently limited range for NO_3^- analysis, the reflectometer has suitable analytical accuracy and practical characteristics to be a useful tool for

improving N management in horticultural farms by enabling rapid and accurate on-farm monitoring of NO_3^- in fertigation nutrient solutions, plant sap and soil solution. Analyses of plant sap and soil solutions require that appropriate adequacy levels be defined for specific cropping situations. Hochmuth (1994) developed sufficiency ranges for NO_3^- in petiole sap for range of vegetable crops. Hartz *et al.* (1993) and Burt *et al.* (1995) provide some guidelines and discussion on NO_3^- sufficiency ranges for root zone soil solution analysis. Granados *et al.* (2007) used a sufficiency range of 8–12 mmol L^{-1} for NO_3^- in the root-zone soil solution to provide corrective N management to a pepper crop. In drip-irrigated vegetable production systems in greenhouses, soils are generally maintained at moisture contents close to Field Capacity; therefore, fluctuations in soil moisture will have little effect on soil solution nitrate concentration. Using on-farm analyses rather than laboratory analysis will facilitate the use of these crop and soil monitoring practices; however, periodic laboratory testing should also be used to verify the results from rapid analysis systems.

These regular crop and soil monitoring procedures are well-suited to horticultural systems where manure applications are often made, as occurs in Almería (Thompson *et al.*, 2007b). This is because they can respond to N recently mineralised from organic N supplied in manure. Such N is not measured in conventional soil mineral N analysis that are normally conducted prior to planting or early in the crop cycle.

The current study demonstrated that the RQFlex reflectometer can provide rapid and accurate analysis of NO_3^- in the soil solution of the root zone and in plant sap. Given that these parameters are sensitive to crop N management, there appears to be appreciable potential for the incorporation of on-farm measurement using the reflectometer or similar systems into corrective N management practices.

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