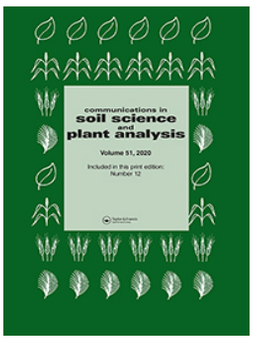

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Rapid Sap Nutrient Analysis Methods in *Malus Domestica* Borkh Cv. 'Gala'

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ABSTRACT

Sap quick tests are well established in vegetable crops. However, there is less equivalent investigation for perennial crops, such as apple trees. Accessing the nutrient content, as opposed to the foliar analysis, would increase the opportunity of adjusting the fertilization, along the growing cycle. This work evaluates the relation between the NO_3^-, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-} and NH_4^+ contents in apple petiole sap, measured with the RQflex® test strips and laboratory methods to assess the possibility of using this tool with accuracy in *in-situ* sap analysis. Petiole samples were collected from six apple tree orchards, frozen and pressed. Dilution was mandatory for all nutrient determination, except NO_3^-. The dilution factor varied with the stage of the annual cycle. The levels of NO_3^-, K^+, Mg^{2+}, and Ca^{2+} followed the same pattern during the growing cycle, with both methods. Regression analysis resulted in high determination coefficients for NO_3^- ($R^2 = 0.85$), K^+ ($R^2 = 0.86$), Mg^{2+} ($R^2 = 0.81$) and Ca^{2+} ($R^2 = 0.95$), between RQflex® and laboratory methods. No equivalent relation was found for ammonium and phosphate determination. These tests can be useful tools for rational fertilization management, mainly in high-density apple orchards. The calcium content in 45 DAFB leaves correlated well with the calcium content in sap at the same timing.

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KEYWORDS
Calcium; magnesium; nitrate; potassium; diagnostic methods

Introduction

Traditionally, leaf analysis is used for the diagnosis of plant nutritional status. Leaf and soil analyses along with yield are used for fertilization recommendation. However, foliar analysis indicates an average value from the beginning of the growth cycle until the time the sample is taken and consequently many effects overlap and make the knowledge of cause/effect relations difficult (Cadahía 2008). Despite being a very good tool to detect deficiency and/or excess of nutrients along with the soil samples analyzed from the same site (Milošević and Milošević 2017), this analysis is costly and time consuming (Gangaiah et al. 2016). Monitoring crop and soil nutrient status with quick analysis is a favorable approach for corrective management (Thompson et al. 2009).

The sap composition includes all the extractable liquids from the conductive tissues, both xylem and phloem (crude and elaborated flow) (Cadahía 2008). It can suggest the nutritional problem quickly and lead to an immediate correction or adjustment. This makes the dynamic study of plant nutrition possible (Cadahía and Lucena 2005; De Souza et al. 2012) because the sap mineral composition is very sensitive to changes and fluctuations, depending on the extraction method: time of day, sampled organ and relative position in the plant, fertilization/irrigation, and atmospheric conditions (Cadahía 2008).

Sap quick tests in vegetable crops are well established, such as potato (Errebhi, Rosen, and Birong 1998; Vitosh and Silva 1996), cabbage (Scaife and Stevens 1983), cauliflower (Kubota et al. 1996), bell

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pepper (Olsen and Lyons 1994), artichoke (Rodrigo et al. 2007), tomato (Folegatti et al. 2005; Hochmuth 1994; Thompson et al. 2009), broccoli (Hochmuth 1994; Kubota et al. 1997) and pumpkin (Studstill et al. 2003). However, there is less equivalent investigation on the use of sap nutrient quick tests for perennial crops, such as apple trees.

The majority of plant analysis is performed by inductively coupled plasma optical emission spectroscopy (ICP-OES) or by inductively coupled plasma mass spectrometry (ICP-MS) (Hansen et al. 2013). The laboratory method is always destructive and implies dissolving the sample in at least one acid (Gangaiah et al. 2016), usually nitric acid.

RQflex® test strips are briefly dipped into a sap sample and allowed to develop color during a standard interval of time, the color intensity is quickly measured using the RQflex® reflectometer (Merck, Darmstadt, Germany). The amount of light reflected from the test strip is measured and then converted to concentration by a standard calibration done previously with a barcoded plastic strip. The measured concentrations are given in mg nutrient L^{-1}. Due to the high concentration of the sap, test strips, and ion-specific electrodes (ISEs) measurements are very affected by other present ions/compounds. The ISEs measurements are accepted in horticulture for petiole sap because the analytical variability of the method (meter-to-meter and interferences) tends to be smaller compared with the biological variability (Di Gioia et al. 2010). Besides the presence of interference elements, the RQflex® reflectometer analytical range is restrictive, implying the dilution of sap before measuring (Parks, Irving, and Milham 2012).

Nagarajah (1999) reported that is possible to use Merck RQflex® test strips as an inexpensive method for monitoring nitrogen levels in Sultana grapevines. The tests done with perennial crops sap, especially for NO_3^- and K^+ measurements (De Souza et al. 2012) imply an extraction with ether, to deal with the chlorophyll, and a subsequent filtration by a decantation process as described in Cadahía (2008). Samples are filtered and treated with activated carbon to reduce interferences with color measurement (Thompson et al. 2009) or by adding ascorbic acid (1%) to reduce oxidation. Preparing solutions that completely eliminate interferences by adding $Al_2(SO_4)_3$ (eliminates organic elements), NH_2HSO_3 (eliminates NO_2^-), or adjusting pH with NaOH (Cadahía 2008; Errebhi, Rosen, and Birong 1998), makes the procedure hard to be used on the field. Beside the interference problems, the sap sample preparation has to be done carefully, for example, the petiole sap nitrate concentration is drastically altered due to the sample-handling procedures – ranging from 800 to 1570 N-NO_3 mg L^{-1}, for petioles stored in polyethylene bag on ice for 6 h and petioles from whole leaves after storage in uncooled, open polyethylene bag on ice for 2 h, respectively (Hochmuth 1994). It is essential to register the phenological stage at sampling, since the sap mineral composition changes drastically along the growing cycle (Cadahía 2008) as well as any fertirrigation intervention and the environmental conditions.

Calcium is regarded as one of the most important mineral elements determining fruit quality. Increasing the calcium content of apple fruit reduces the postharvest decay (Conway, Sams, and Hickey 2002), especially in fruits that will be stored for a long time, such as the case of apples and pears (Faust 1989). Higher calcium concentration is a pre-requisite for the lower incidence of calcium-related diseases and improved fruit nutritional value (Montanaro et al. 2014). Therefore, the ratios N/Ca^{2+}, K^+/Ca^{2+}, and $(Mg^{2+} + K^+)/Ca^{2+}$ need to be optimized for storage (Amiri, Fallahi, and Golchin 2008). Many internal and external factors affect the calcium uptake by roots and its transport to leaves and fruits, such as the transpiration rate (Murtić et al. 2017). However, leaf Ca^{2+} concentration is not a good predictor for fruit Ca^{2+} levels. Many factors affect Ca^{2+} accumulation in fruit: shoot and fruit growth rate, B availability, level of Mg^{2+} and Zn^{2+}, and soil moisture (Faust 1989). The K^+ content in fruits is directly related to their carbohydrate content, mainly in highly productive apple orchards (Brunetto et al. 2015; Faust 1989) and it has a major role determinant for protein synthesis (Faust 1989). A good predictor or indicator of the Ca^{2+}, K^+ and Mg^{2+} fruit content, early in the growth cycle, allowing to adjust fertirrigation may improve the control of fruit storage physiological disorders.

The objective of this research is to evaluate the relation between the NO_3^-, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, and P contents in apple petiole sap, measured with the RQflex® test strips and laboratory methods

(ICP-OES and VIS spectrophotometry), to assess the possibility of using this tool with accuracy in *in-situ* sap analysis. This would help growers and technicians to adjust fertilizer inputs according to apple tree needs.

Material and methods

Experimental layout, plant material and environmental conditions

The orchards were located at the Experimental Farm of Lisbon University (A) and at five private orchards (B, C, D, E and F) representative of the production area, Alcobaça. Location details, spacing, and planting dates can be found in Table 1.

The trials were carried out in 2019 on 'Gala' grafted on M9 and central leader trained. In all orchards, trees were pruned, fertilized, irrigated, and protected from pests and diseases according to local commercial practice. The trees selected for sampling had homogenous vegetative growth and flower intensity.

Leaf sampling

In orchard A, in five sampling dates, between April 22 (Biologische Bundesanstalt, Bundessortenant und Chemische Industrie (BBCH) 65) and September 16 (BBCH 91), 147 days after full bloom (DAFB) whole full green leaf samples, from the medium third, were collected. The sampling was done between 08:00 am and 09:30 am, on sunny days, to reduce the effect of diurnal nutrient variations, reported for Sultana grapevines by Nagarajah (1999).

All tests were done with three biological replicates and each one was composed of 120 whole leaves (blade and petiole) removed from 15 trees (8 leaves per tree). The leaves were also sampled in the B, C, D, E, and F orchards in two dates: 45 and 90–110 DAFB.

To the sap analysis procedure, leaves were collected fully green, totally developed, but not in senescence, at the same height in the tree or similar leaf position, as much as possible to reduce variations (Faust 1989). Then, the leaves were transported in a closed polyethylene bag to the laboratory, where the petioles were separated from the blades, cut in 0.5 cm pieces, weighted, and immediately stored in a − 20°C freezer, for at least 24 h until the measurements. The Alcobaça leaf samples were transported to Lisbon stored in thermal bags with ice and were processed on the same day.

Freezing pre-treatment

The 120 cut petioles were stored in plastic test tubes in the freezer. This helped the sap extraction process due to cell rupture. The sap was much more easily extracted resulting in more mL of sap extracted for the same petiole quantity. Furthermore, freezing petioles prior to extraction significantly increases the release of both nitrate and potassium from the petioles as reported by Nagarajah (1999).

Table 1. Orchard locations, spacing and planting dates.

Orchard	Location	Latitude	Longitude	Spacing (m)	Planting Date
A	Lisboa	38° 42 27.5″ N	9° 10′ 56.3″ W	4.0 x 1.0	2016
B	Alcobaça	39° 28 30.48″ N	9° 07′ 12.72″ W	4.0 x 1.0	2015
C	Alcobaça	39° 30 55.01″ N	9° 00′ 54.71″ W	3.3 x 0.85	2016
D	Alcobaça	39° 32 55.36″ N	8° 57′ 22.52″ W	4.5 x 1.2	2004
E	Alcobaça	39° 35 20.06″ N	8° 59′ 11.40″ W	3.8 x 0.8	2015
F	Alcobaça	39° 26 59.95″ N	9° 1′ 5.14″ W	3.8 x 0.7	2016

Sap extraction

The petioles were manually crushed in a hydraulic press, the sap was then collected and its volume measured. In order to avoid sap oxidation measurements with RQflex® test strips, preparation for the laboratory analysis was done immediately.

RQflex® reflectometer procedure

Due to the high sap concentration, dilution with water was mandatory for the Ca^{2+}, Mg^{2+}, and K^{+} determination, so that the concentration of the diluted sample was mid-way within the analytical range. The required dilution factor depends on the moment in the growing cycle. In April, at full bloom in orchard A (F65 BBCH), the required dilution factor was 5X for Ca^{2+} and Mg^{2+} and in September was 10X, for both. The same happened for the K^{+} tests, from 10× to 20 ×.

The N-NO_3^- measurement was made using non-diluted fresh sap.

ICP-OES sap sample preparation

The sap samples were diluted 20X in an acid nitric solution (5%) and stored in a conventional freezer to be analyzed by ICP-OES (Unicam) and determine Ca^{2+}, Mg^{2+}, K^{+}, and total P content (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The supernatant was used for the analysis.

VIS spectrophotometry sap sample preparation

The sap sample was diluted 20× with KCl (2 M) to the N-NH_4^+ and N-NO_3^- determination and then analyzed by VIS spectrophotometry (Skalar Analytical B.V., Breda, Netherlands) using Berthelot and sulfanilamide methods (Houba et al. 1989).

Calibration with standard solutions – RQflex® tests, VIS spectrophotometry and ICP-OES correlations

To evaluate the analytical accuracy of the RQflex® method, previous calibrations with prepared standard solutions were tested, by comparing the test strips results with ICP-OES and VIS spectrophotometry. The RQflex® Ca^{2+}, K^{+}, Mg^{2+}, and PO_4^{3-} test strips were tested against the ICP-OES measurements using KNO_3, $Mg(NO_3)_2$, $Ca(NO_3)_2$, and KH_3PO_4 standard solutions. Standard solutions were prepared accordingly to the RQflex® potassium, calcium, magnesium, and phosphate test strips analytical range: 0.25–1.20 g K^{+} L^{-1}, 5–100 mg Mg^{2+} L^{-1}, 5–125 mg Ca^{2+} L^{-1}, and 5–120 mg PO_4^{3-} L^{-1}, according to the manufacturer.

To access the relation between the RQflex® NO_3^- and NH_4^+ test strips and VIS spectrophotometry, KNO_3 and NH_4Cl solutions diluted in KCl (2 M) through the analytical test range 5–225 mg NO_3^- L^{-1} and 0.2–7 mg NH_4^+ L^{-1}.

Mineral foliar analysis

For P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B determination, 0.30 g of dried crushed whole leaves were digested in a mix of HCl and HNO_3, following an adapted version of the European Norm EN 13650 (CEN 2001) and analyzed by ICP-OES.

To N determination, 0.25 g of dried crushed leaves were digested in 4 mL of H_2SO_4 with selenium as a catalyzer, following the Kjeldahl method (Horneck and Miller 1998). The samples were analyzed by VIS spectrophotometry using the Berthelot method (Houba et al. 1989).

Statistical analysis

Data were analyzed by conducting regression analysis between the nutrients measured by laboratorial methods and the RQFlex® quick test determinations. Statistical significance was assessed by Student's t-tests, whenever assumptions were proven and the null hypothesis was rejected at $\alpha = 0.05$. Statistical analyzes were conducted in R software (R Core Team 2019).

Results

Sap extraction

From the 120 collected petioles per sample in orchard A was possible to extract between 2.6 and 3.6 mL of sap, during the growing cycle (Table 2), this amount of sap was enough for the two tested methods.

Calibration with standards

The RQflex® K^+, Ca^{2+}, Mg^{2+}, and P on ICP-OES regressions, with the described standard solutions, all presented an $R^2 > 0.99$ (N = 10). The relation between RQflex® N-NO_3^- and N-NH_4^+ and the VIS spectrophotometry presented an $R^2 \approx 0.99$ (N = 10).

N-NO_3^- sap analysis

The RQflex® nitrate measurements were converted to N units to be comparable with VIS spectrophotometry. During the evaluated time, the N-NO_3^- sap concentration, in orchard A, was higher in 91 and 147 DAFB. The other three tested moments share similar concentrations and non-significate differences. The decrease in N-NO_3^- sap content, from the 91 to the 129 DAFB, was supported by both methods (Figure 1a). To low N-NO_3^- concentrations, the data points spread, suggesting a worse adjustment for lower values. Such finding was reported as well by Nagarajah (1999) in the sap of Sultana grapevines (Figure 1b).

N-NH_4^+ sap analysis

RQflex® NH_4^+ results were converted to N-NH_4^+ to be comparable with the VIS-spectrophotometry measurements. No relation was found between these methodologies (Figure 2b). The quick test reports a very similar N-NH_4^+ concentration during the tested period, detecting almost no difference, and the laboratorial method marks the 45 and 91 DAFB as the most concentrated in this element (Figure 2a).

Phosphorus sap analysis

The RQflex® measures P contents in the form of phosphate and the ICP-OES measures P, to compare these results, PO_4^{2-} was converted to mg P L^{-1}. The relation found between the laboratorial method and the quick test was not acceptable as shown in Figure 3b. The P sap content reached the minimum value in the 91 DAFB timing (Figure 3a).

Table 2. Collected quantity of fresh petioles (g) and extracted sap (mL), mean ± SE between 28 and 147 DAFB, for orchard A (n = 3).

DAFB	Fresh petioles (g)	Sap (mL)	Sap/fresh petioles (mL g^{-1})
28	11.6 ± 0.46	3.2 ± 0.21	0.27
45	10.8 ± 0.39	2.6 ± 0.15	0.24
91	11.2 ± 0.72	3.1 ± 0.06	0.27
129	11.9 ± 0.39	2.9 ± 0.22	0.25
147	13.6 ± 0.70	3.6 ± 0.11	0.26

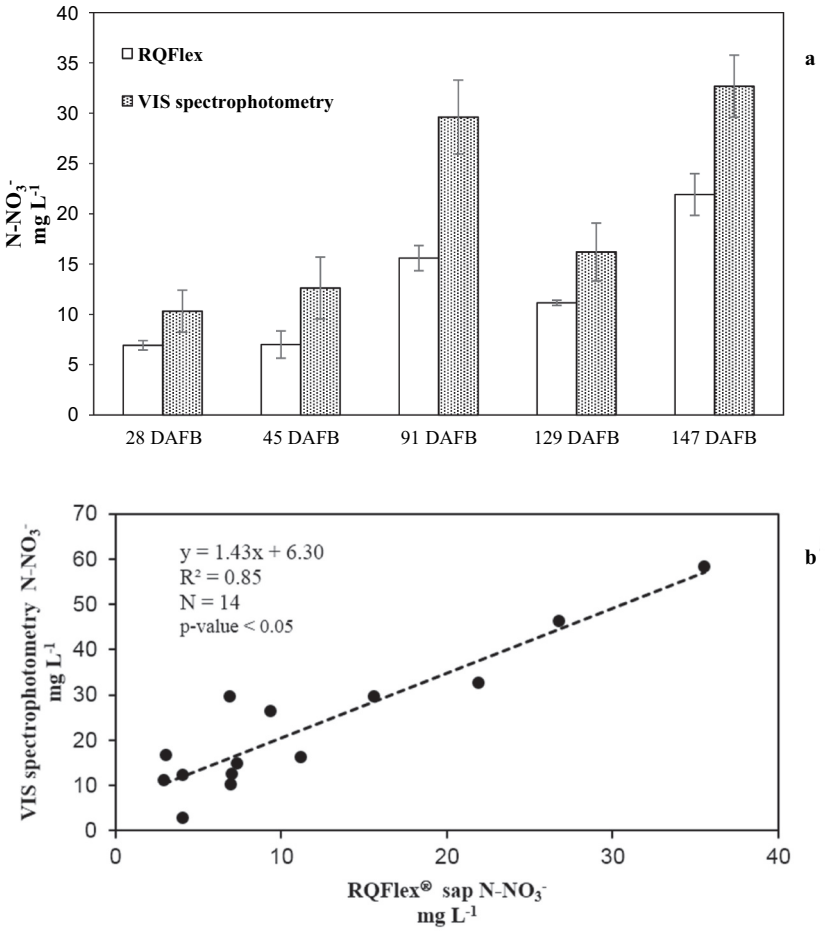

Figure 1. Evolution of sap $N\text{-}NO_3^-$ content measured by RQflex® test strips and VIS spectrophotometry (a), from 28 to 147 DAFB, for orchard A and regression of VIS spectrophotometry on RQflex® sap $N\text{-}NO_3^-$ test (b) with data from all orchards. Bars are mean ± standard deviation.

Potassium sap analysis

The K^+ sap content followed a similar evolution, measured with the RQflex® test strip and by ICP-OES (potassium). At 45 DAFB the K^+ concentration (9.73 and 9.53 g L^{-1}) was maximum, the minimum (5.87 and 5.97 g L^{-1}) happened at the 147 DAFB, for RQflex® and ICP-OES, respectively (Figure 4a). The relation between the K^+ contents measured with test strips and by ICP-OES, using data from all tested orchards, was well correlated (R^2 = 0.86), especially for mid-range values (7.5 to 9.0 g L^{-1}) (Figure 4b).

Calcium sap analysis

The Ca^{2+} sap content increased slightly after 91 DAFB (Figure 5a). The regression of RQflex® test strips on ICP-OES correlates well (R^2 = 0.95). Although to higher concentrations (1000 mg Ca^{2+} L^{-1}) the strength of the relation decreases, suggesting a poorer linear adjustment when the Ca^{2+} content is high (Figure 5b).

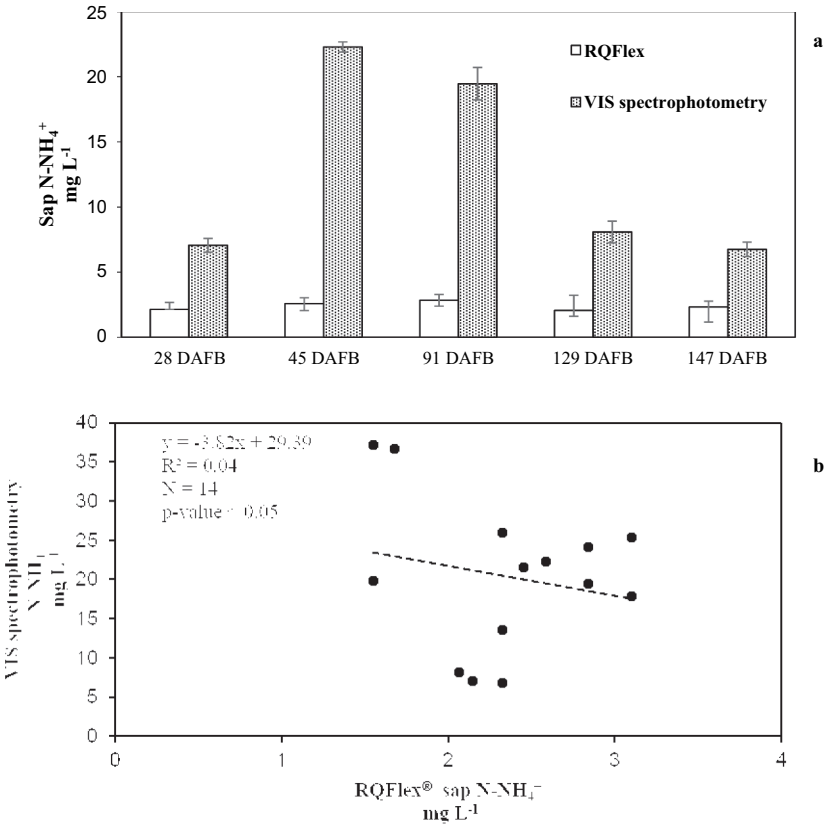

Figure 2. Evolution of sap N-NH_4^+ content measured by RQflex® test strips and VIS spectrophotometry (a), from 28 to 147 DAFB, for orchard A and regression of VIS spectrophotometry on RQflex® sap N-NH_4^+ test (b) with data from all orchards. Bars are mean ± standard deviation.

Magnesium sap analysis

The Mg^{2+} sap content followed a similar pattern to the Ca^{2+} concentration, along the cycle (Figure 6a). The minimum, at 45 DAFB, is not significantly different from 28 DAFB. After the 91 DAFB, the sap content increases to a maximum of 1271.6 mg Mg^{2+} L^{-1}, as measured by ICP-OES. The correlation between the Mg^{2+} measured values with the RQflex® test strips and by ICP-OES was R^2 = 0.82 (Figure 6b).

Foliar mineral analysis at 45 and 90-110 DAFB

According to the nutritional reference values for leaves at 90–110 DAFB, for 'Gala' (INIAP-Laboratório Químico Agrícola Rebelo da Silva 2006), the concentration of N, K, S, and B is less than ideal to the trees nutritional balance – this is especially relevant for N content, since the gap between the leaf concentration and the reference is 0.8% (Table 3).

Relation between leaf and sap calcium content for 45 and 90 DAFB

The leaf Ca content determined by conventional laboratory method correlates with the same nutrient measured in the sap, at 45 DAFB (R^2 = 0.59) and at 90–110 DAFB (R^2 = 0.37), using data from all

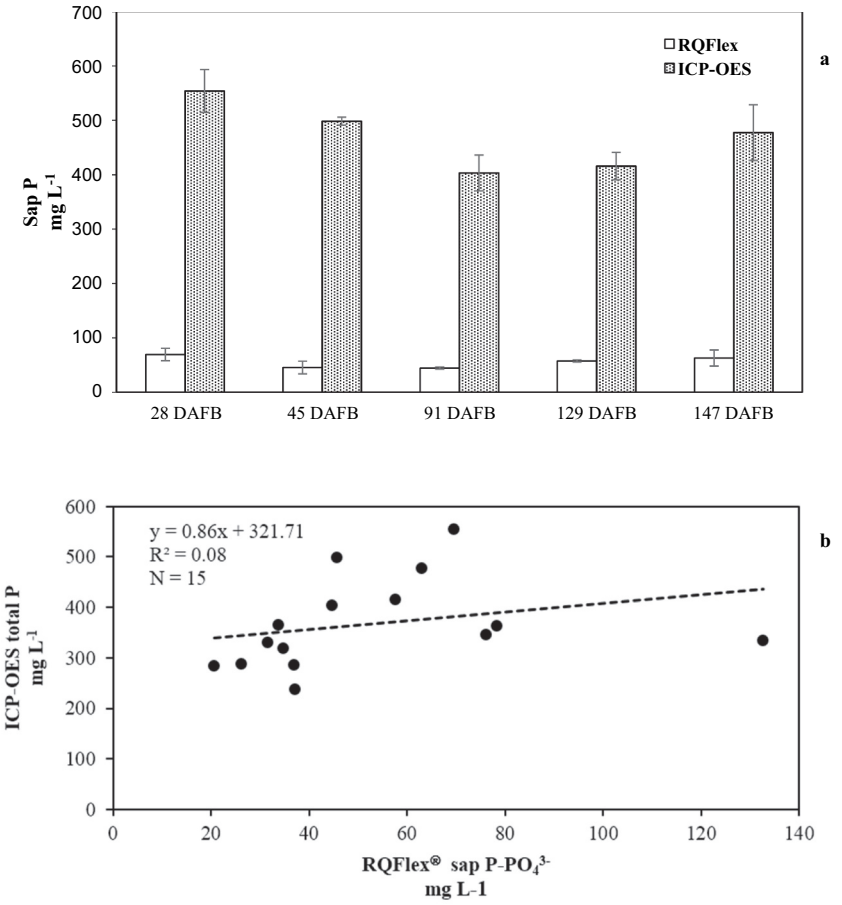

Figure 3. Evolution of sap P content measured by RQflex® test strips and measured by ICP-OES (a), from 28 to 147 DAFB, for orchard A and regression of VIS spectrophotometry on RQflex® sap *P*-PO_4^{3-} test (b) with data from all orchards. Bars are mean ± standard deviation.

orchards. Merging data from 45 and 90–110 DAFB, the linear regression between leaf and sap Ca improves its quality ($R^2 = 0.83$) (Figure 7). Conventional leaf calcium content and sap Ca^{2+} measured by the Rqflex® method showed an $R^2 = 0.73$.

Table 3. Macro (%) and micronutrients (mg kg^{-1}) analysis at 45 and 90–110 DAFB, mean ± SE, for orchard A (n = 3), and nutrient reference values for 'Gala' (INIAP-LQARS, 2006). Values reported to dry weight.

Nutrient	Unit	45 DAFB	90–110 DAFB	Reference values 90–110 DAFB
N	%	2.96 ± 0.50	1.70 ± 0.43	2.53–3.00
P	%	0.24 ± 0.01	0.27 ± 0.02	0.14–0.18
K	%	1.41 ± 0.02	1.36 ± 0.06	1.37–2.00
Ca	%	0.81 ± 0.02	1.20 ± 0.05	0.90–1.45
Mg	%	0.29 ± 0.01	0.35 ± 0.02	0.20–0.30
S	%	0.17 ± 0.01	0.19 ± 0.01	0.22–0.32
Fe	mg kg^{-1}	101.31 ± 10.69	377.23 ± 64.28	> 40
Mn	mg kg^{-1}	49.38 ± 8.25	54.31 ± 5.95	50–200
Zn	mg kg^{-1}	37.13 ± 2.97	51.89 ± 1.88	10–50
Cu	mg kg^{-1}	10.53 ± 0.77	13.68 ± 1.30	7–14
B	mg kg^{-1}	14.73 ± 1.20	24.18 ± 1.34	25–35

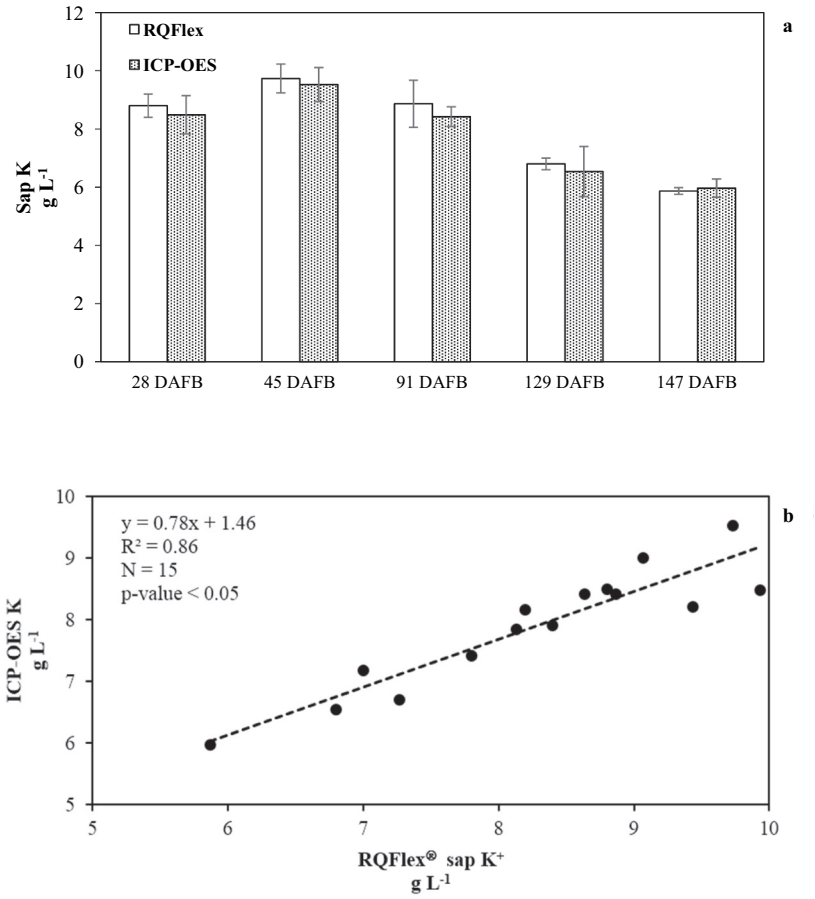

Figure 4. Evolution of sap K^+ content measured by RQflex® test strips and potassium measured by ICP-OES (a), from 28 to 147 DAFB, for orchard A and regression of ICP-OES on RQflex® sap K^+ test (b) with data from all orchards. Bars are mean ± standard deviation.

Discussion

The RQflex® reflectometer is not meant to replace the laboratorial methods. There are many affecting factors, as the presence of interfering ions, the sap color, and the oxidation/reduction reactions that take place as soon as the petioles are crushed and the sap is exposed to the air. In this work, no pre-treatment was done to test the possibility of a simple method for quick and sufficient approximation to the sap mineral contents. The test strips method implies a pre-freezing treatment at least overnight and sap dilutions for each tested nutrient. The dilution requirement is even more critical in high production orchards, with increased fertilization programs: such as the orchards B, C, D, E, and F. Nevertheless, the flexibility of storing samples in a freezer and completing the sap test later is an important practical advantage, since technicians can schedule the analysis for a convenient time.

These tests did not work with phosphorus and ammonium, since the relationship was not satisfactory between the strips and the laboratorial measurements. In sap, P is present as inorganic phosphate, which can be quantified by quick test strips, yet it is also found in multiple organic compounds in plant metabolism and as a constituent of most enzymes (Marschner 2012). ICP-OES destroys all the sap chemical structures, since the Ar plasma temperatures can range from 7480 to 7910 K (Scheffler and Pozebon 2015) or even greater which leads to higher concentrations of P in sap, compared with the low concentrations determined by the quick tests that only quantify P in the

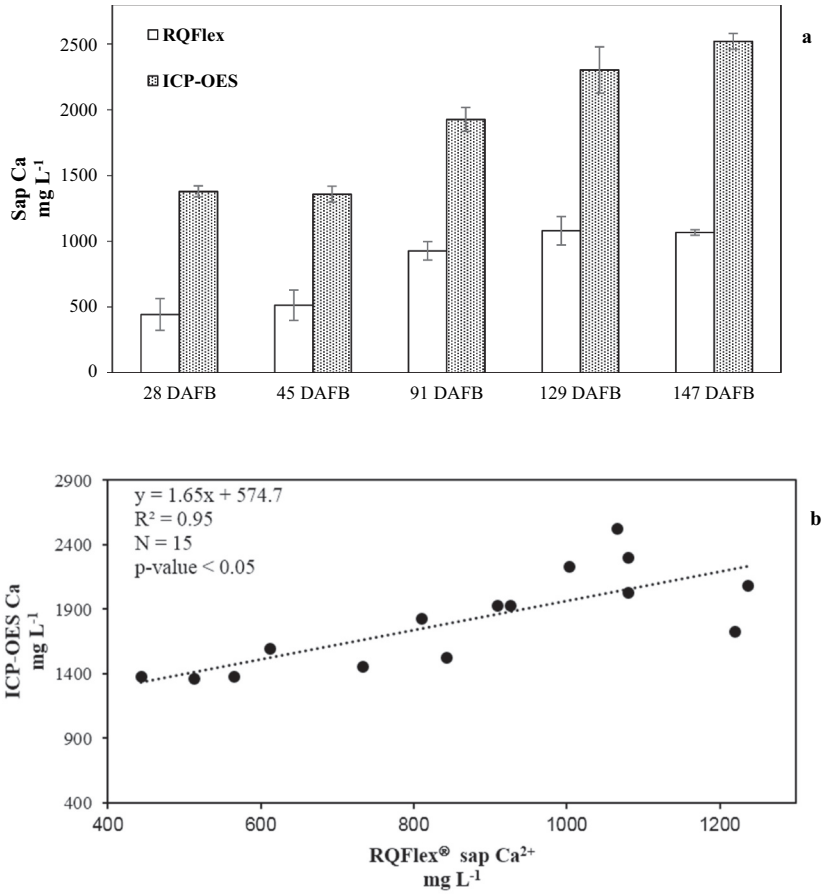

Figure 5. Evolution of sap Ca^{2+} content measured by RQflex® test strips and ICP-OES (calcium) (a), from 28 to 147 DAFB, for orchard A and regression of ICP-OES on RQflex® sap Ca^{2+} test (b) with data from all orchards. Bars are mean ± standard deviation.

phosphate form. Besides, the proportion of organic and inorganic P sap forms is variable and the ICP-OES P measurements suggest a greater variation of the organic P forms. The N-NH_4^+ sap content quick test determination is particularly sensitive to the presence of Fe^{2+} and Fe^{3+}, according to the technical info provided by the manufacturer, which can explain the low-quality relation between the VIS-spectrophotometry N-NH_4^+ determinations and the quick test strips method. Besides, in well-aerated soils NO_3^- is the predominant N source for tree uptake (Tromp and Ovaa 1979). Thus, NH_4^+ is less likely to be taken up, translocated through the xylem, metabolized and reduced to NO_3^-, and allocated to the apple tree organs, generating lower concentrations of this element and consequently harder to measure.

Both leaves and fruit (60 DAFB and 2 weeks prior to harvest) were analyzed for minerals (data not shown) but no relation was found between these measurements as reported by Faust (1989). Nagarajah (1999) has reported a relationship between minerals in dried petioles conventional analysis and sap at flowering in Sultana grapevines. Our results support a relation between the sap Ca^{2+} content and the conventional leaf analysis, especially for the 45 DAFB timing (R^2 = 0.59). The sap Ca^{2+} variation range, besides the analytical precision, comes from the diverse orchard management systems implemented, soil and plant conditions, ranging from 1300 to 1800 mg Ca^{2+} L^{-1} in this timing. Correlating sap Ca^{2+} content, as measured by RQflex® test strips, with the laboratorial method and with the leaf Ca content can be used as an early indicator of the reference

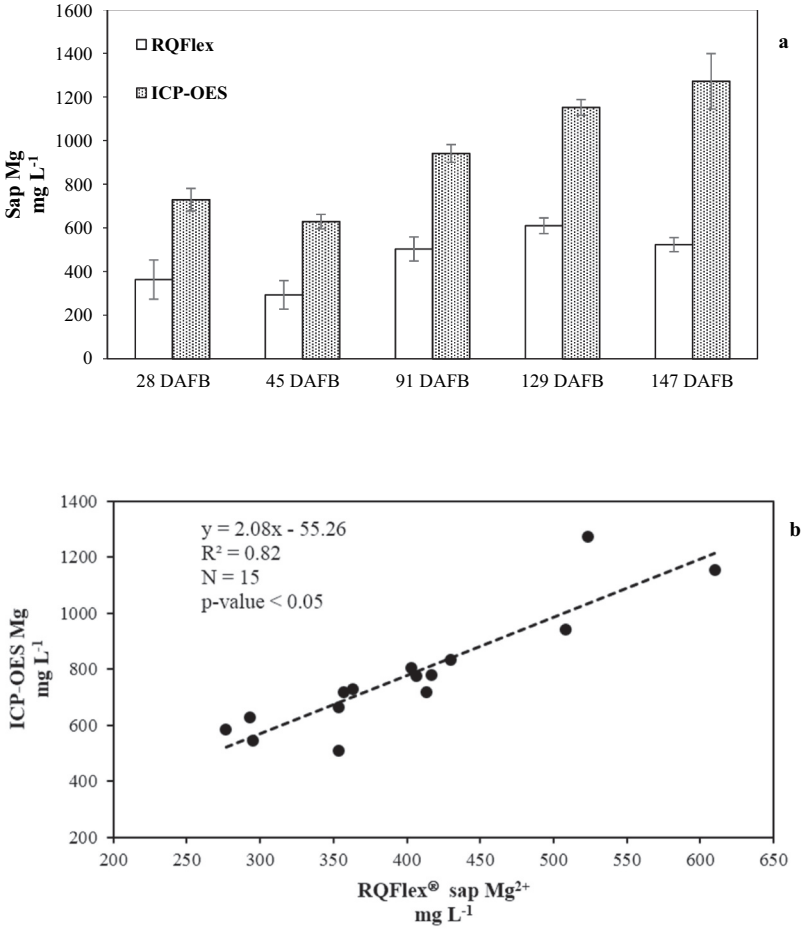

Figure 6. Evolution of sap Mg^{2+} content measured by RQflex® test strips and ICP-OES (magnesium) (a), from 28 to 147 DAFB, for orchard A and regression of ICP-OES on RQflex® sap Mg^{2+} test (b) with data from all orchards. Bars are mean ± standard deviation.

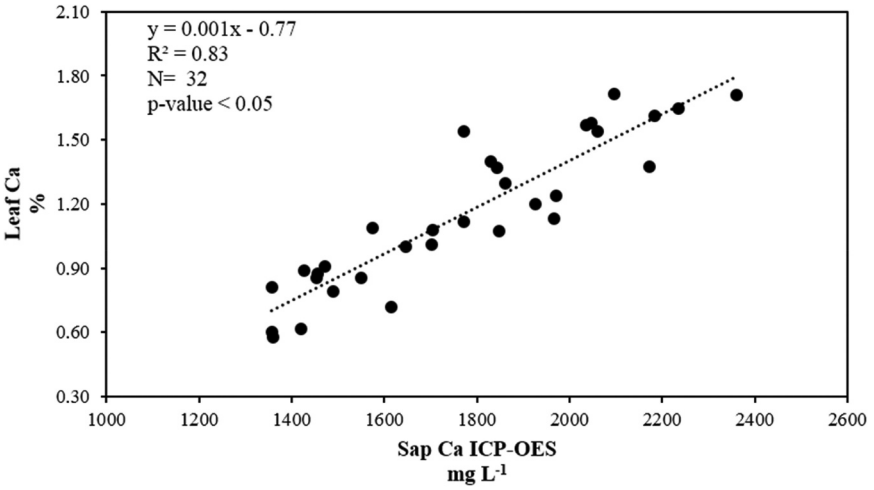

Figure 7. Linear regression of sap Ca measured by ICP-OES on leaf Ca content, using data from all orchards and both timings.

Ca leaf content, suggesting a more rational fertilization approach in the orchards, especially for 45 DAFB, since the slope for the 90–110 DAFB linear regression is almost flat. The same was done to K^+, Mg^{2+} but since the range of variation was smaller, these nutrient contents were similar for all orchards. The variability in the measurements was due to the analytical error more than due to the differences in orchards. The N-NO3- quick measurement can lead to a greater general awareness of the nitrogen levels in the sap.

Over the last years, several models based on both leaf and fruit minerals to predict pre-harvest fruit quality were proposed (Fallahi et al. 2010; Jivan and Sala 2014). These models correlate N, P, K^+, and Ca^{2+} with fruit quality, disorders, and/or yield. However, there are no validated standards for sap nutrient content in apple trees and it is necessary to make field trials aiming to provide information on variation in leaf and sap nutrient concentration related to yield and fruit quality. This strip quick test may produce data that will enable us to build models for fruit yield and quality prognosis.

Fruit and sap RQflex® analysis along the fruit development would be of practical interest, in order to study the possibility of using the K^+, Ca^{2+}, and Mg^{2+} sap contents as a fruit quality predictor. Analyzing sap mineral content is a way to detect sensitive changes in the tree's nutrition comparing with leaf analysis. However, apple tree sap standards are needed to improve the quality of the information obtained. Nutrient standards for apple tree sap would give an immediate feedback and facilitate adjustments in the fertilization program as a fertilization index. This work already exists for: *Olea europaea* L., *Vitis vinifera* L., *Prunus persica* L., and *Citrus* (Cadahía 2008).

Conclusions

The RQflex® Ca^{2+}, Mg^{2+}, K^+ test strips for sap analysis correlated well with the laboratory methods and can be a useful management tool to monitor seasonal nutrient demands increasing apple fruit quality. The calcium content in 45 DAFB leaves correlated well with the calcium content in sap at the same timing. In intensive production systems, where the inputs of nitrogen are generally high, the RQflex® can be used to measure NO_3^- sap concentrations allowing more rational nutrient management with environmental advantages.

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