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Research Paper

Estimation of plant nutritional status by Vis–NIR spectrophotometric analysis on orange leaves [*Citrus sinensis* (L) Osbeck *cv* Tarocco]

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Nutritional status in citrus plants, which is used as a guide for fertilisation, is normally determined by chemical analysis of leaves. According to standardised procedures, this is a destructive method. Leaf analysis detects symptomless detrimental conditions or confirms the nature of visible toxicity. This study proposes the use of a rapid, non-destructive, cost-effective technique to predict orange leaves nutritional status utilising a Vis–NIR (visible–near infrared) portable spectrophotometer and compares its results with standard chemical analyses. Tree nutritional status was evaluated by foliar analysis performed on 50 leaves. Chemical determinations on leaves detected N, P, K, Ca, Mg, Fe, Zn, Mn. For spectral acquisition, a 'pen probe' was used to measure the spectral reflectance response on each leaf. Mean reflectance values of all leaves for each treatment were compared by chemometric multivariate methods (PLS, partial least square) to both: a single reference chemical value and to all chemical parameters used together. The best model for single reference chemicals (coefficient of correlation $r = 0.995$) and the tests ($r = 0.991$) was obtained for potassium. Results also showed a high efficiency in the determination of nitrogen. For all chemical parameters used together, the analysed elements gave correlations in a range from $r = 0.883$ for Mg to $r = 0.481$ for P with standard error of prevision ranging from 0.01 for P to 12.418 for Fe.

1. Introduction

There is an increasing need to review information on crop nutrition to adequately establish nutrient requirements and to fine-tune fertiliser rates. This is due to the need to optimise fertilisation programmes in order to maximise the yield of high quality fruit (Embleton, Coggins, & Witney, 1996; Embleton, Reitz, & Jones, 1973a; Koo, 1989; Legaz-Paredes & Primo-Millo, 2000), whilst minimising the amount of chemical fertilisers applied, to reduce the risks of environmental impact (Alva, Paramasivam, Graham, & Wheaton, 2003; Davies, 1997).

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Nomenclature

ALL	All the reference chemical values used together for the chemometric analysis.
LV	Latent variables.
PLS	Partial least squares.
R^2	Coefficient of determination.
RMSE	Root mean square error.
RMSEC	Root mean square error in calibration.
RMSECV	Root mean square error in cross validation.
SEP	Standard error of prevision.
SINGLE	Single reference chemical value used for the chemometric analysis.
Vis–NIR	Visible – near infrared.

Nutrients are essential for the proper metabolic functioning of trees and to ensure desirable commercial production (Davies & Albrigo, 1994). They vary considerably with citrus-growing region, soil type, cultural techniques, leaf age and position on the tree, age of the tree and rootstock/scion combination. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) are macro, meso and micronutrients of citrus leaves associated to growth, yield and quality factors, with relationships that vary with different elements (Embleton, Reitz *et al.*, 1973b; Hanlon, Obreza, & Alva, 1995).

Leaf analysis is the most important tool for evaluating nutrient status of citrus and for guiding its fertilisation. Although other organs within the plant may act in a similar manner, the leaf is the most readily available source of tissue for analysis, it is metabolically very active, being the site of photosynthesis which determines the primary processes occurring within the plant, and the leaf is a major site of carbohydrate and mineral storage (Embleton, Jones, Labanauskas, & Reuther, 1973a). Analytical evaluations are performed more frequently, and with different aims, directly on the fruit as reported by much research (Cayuela, 2008; Gomez, Yong, & Pereira, 2006; McGlone, Fraser, Jordan, & Kunnemeyer, 2003; Steuer, Schulz, & Lager, 2001).

Results of the chemical analysis allow interpretation of plant nutritional status, identification of nutrient disorders caused by mineral excess or deficiency, and serve as guide for balanced fertilisation programmes (Ferguson, Davies, Tucker, Alva, & Wheaton, 1995; Intrigliolo, Tittarelli, Roccuzzo, & Canali, 1998; Obreza, Alva, Hanlon, & Rouse, 1992). These analyses are normally compared to well-established standard values referred to as standard age spring-cycle leaves, taken from non-fruiting terminals of mature, fruit-bearing citrus trees. Embleton, Jones *et al.* (1973b) reported the leaf analysis standards for mature, fruit-bearing orange trees based on 5–7 month-old spring-cycle leaves from non-fruiting terminals. The values are within the range varying from deficient to excess categories as suggested by the guidelines for interpretation of leaf analysis (Intrigliolo, Roccuzzo, Lacertosa, Rapisarda, & Canali, 1999; Obreza *et al.*, 1992).

The generalised lowering of the costs of the miniaturised spectrophotometers, provides the possibility of using portable devices directly in the orchard for monitoring the maturity state of fruit. Using instruments directly in-field involves different interferences due to the environmental conditions *e.g.* illuminations (type and strength) and temperatures that should be taken into account during data processing as suggested by Ventura, De Jager, De Putter, and Roelofs (1998). Walsh, Guthrie, and Burney (2000) made an interesting comparative study of the performance of different commercial portable spectrometers to measure the SSC of rockmelons and found some differences in terms of spectral resolution, stability, signal to noise ratio, stability over time and calibration performance. Although such differences were shown, portable spectrometers are still currently used in many applications (Hernandez Sanchez, Lurol, Roger, & Bellon-Maurel, 2003; Miller and Zude-Sasse, 2004; Saranwong, Sornsrivichai, & Kawano, 2003a, 2003b; Temma, Hanamatsu, & Shinoki, 2002; Zude *et al.*, 2006). Another well known and established techniques that must be considered while exploring the nutritional status of a plot of land, is that based on the SPAD Chlorophyll Meter (Lee, Searcy, & Kataoka, 1999; Piekielek, Fox, Toth, & Macneal, 1995; Read, Tarpley, McKinion, & Reddy, 2002), that has as its principle the determination of leaf chlorophyll and thus the estimation of nitrogen content; these being well correlated (Esposti *et al.*, 2003).

Thus, the nutritional status in citrus plants is normally determined by chemical analysis, but in this study a rapid, non-destructive, cost-effective technique to predict orange leaves nutritional status utilising a Vis–NIR (visible–near infrared) portable spectrophotometer is investigated and its results compared with results from standard chemical analyses.

2. Materials and methods

2.1. *Data collection*

The study was conducted on the experimental farm "Palazzelli" of CRA-ACM (Eastern Sicily, 37°17′56″76 N, 14°50′29″76 E), in an irrigated Tarocco blood orange orchard [*Citrus sinensis* (L) Osbeck], planted in a sandy loam soil. Two different clones of Tarocco were tested for leaves nutrient content: "Arcimusa" and "NL Meli" both grafted on sour orange [*C. aurantium* (L.)].

Within the chosen field, using a randomised block design, the trees received different nitrogen treatments to ensure the heterogeneous nutritional status of the leaves tested. Five rates with different nitrogen input levels were applied; 0–200–400–600–800 g N $tree^{-1}$ $year^{-1}$. N was applied as ammonium sulphate; all treatments received the same amount of P (200 g P_2O_5 $tree^{-1}$ as triple super phosphate) and K (350 g K_2O $tree^{-1}$ as potassium sulphate).

Tree nutritional status was evaluated by foliar analysis performed on 50 leaves of the index trees, placed in the middle of the plots. During the month of October, in the external side of the canopy, 5/7-month-old leaves of the year's spring flush were collected from non-fruiting twigs, according to the procedures of Embleton, Reitz *et al.* (1973a) adapted to Italian conditions by Intrigliolo *et al.* (1999). Thirty leaves of each sample were analysed at the chemistry laboratory of the

CRA–ACM (Citriculture and Mediterranean Crops Research Center of the Agricultural Research Council). The remaining 20 leaves, were analysed at the CRA–ING (Agricultural Engineering Research Unit of the Agricultural Research Council) Laboratory using spectrophotometric techniques. The acquisition of the raw spectral curve through the spectrophotometer took about 2 s for each leaf.

2.2. *Chemical analysis*

Chemical analysis from leaves regarded the following elements: N, P, K, Ca, Mg, Fe, Zn, Mn. The leaves were: i) washed in tap water by rubbing both sides using cheesecloth, ii) rinsed in deionised water, iii) oven dried at 65 °C for 72 h, iv) ground and v) dried at 105 °C for 4 h. The concentration of N was determined on 1 g of ground leaf tissue using the micro-Kjeldahl method (Distillation Unit K370, Büchi Analytical Inc., Switzerland). Another 1 g of ground leaf tissue was ashed in a muffle furnace at 550 °C for 12 h. After incineration and extraction with nitric acid (1% v/v) P, K, Ca, Mg, Fe, Zn and Mn were determined using inductive coupled plasma-optical emission spectrometry (ICP-OES; OPTIMA 2000DV, Perkin–Elmer, Italy).

Nutrient concentrations were expressed as a percentage or parts per million (ppm) of the tissue dry matter.

2.3. *Spectrophotometric analysis*

For the Vis–NIR measurements, a (portable) single channel spectrophotometer was used (Fig. 1). The system is composed of five parts: 1) a spectrograph Hamamatsu S 3904 256Q (Hirakuchi, Hamakita-ku, Hamamatsu City, Japan) in a special housing; a customised illumination system realised by a 20 W halogen lamp and an optical fibre bundle consisting of approx. 30 quartz glass fibres; 2) an optical entrance with input round: 70 μm × 2500 μm and diameter 0.5 mm NA = 0.22 mounted in SMA-coupling; 3) specific probes with quartz optical fibre connectors; 4) a transmission device with variable optical length for transmitted or absorbed light from thin solids or liquids; 5) a notebook computer equipped with specific software to acquire, calibrate and elaborate spectral data. The Hamamatsu spectrograph had the following characteristics: grating: Flat-field, 366 L/mm (centre); spectral range: 310–1100 nm; wavelength accuracy absolute: 0.3 nm; Temperature-induced drift: <0.02 nm K^{-1}; resolution (Rayleigh-criterion): DlRayleigh ⟫ 10 nm; sensitivity: ⟫ 1013 Counts/Ws (with 14-Bit-conversion); stray light: <0.8% with halogen lamp and A/D converter 16 bit.

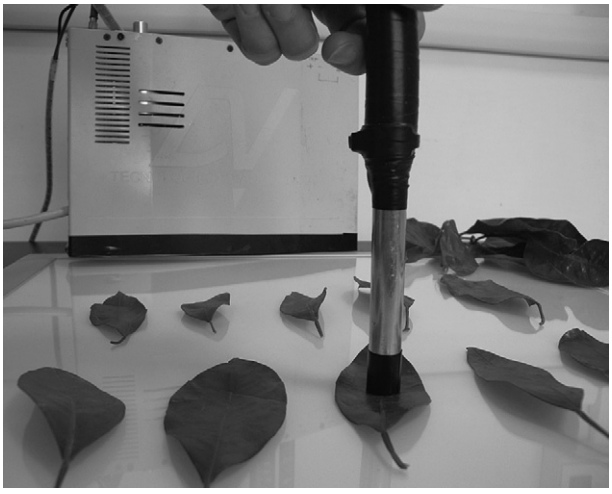

Fig. 1 – Performing VIS–NIR spectral measurement on citrus leaves.

To acquire spectra, the 'pen' probe was used to measure the spectral reflectance response on each single leaf (spot area ≈ 10 mm^2). The reflectance measure is acquired by an optical quartz fibre (0.7 mm in diameter) fixed at 45° inside a circular aperture of 4 mm in diameter. Because the surface of the leaf was soft it was possible to exclude all extraneousness light from the probe.

Spectral measurements were performed in laboratory following a white calibration (small variations in the level of external light), the instrumental integration time (light acquisition time) and subtracting the background noise (variable with the instrument temperature) (Fig. 1). A very low Signal/Noise ratio was observed at the beginning and the end of the spectral data, affecting the accuracy of measurements, hence only spectra in the range 400–1000 nm were taken into account for the analysis. All spectral values were expressed in terms of relative reflectance. After each 30–35 spectral measurements a new white calibration was carried out. The power supplied by the portable batteries of the instrument and the notebook computer, guaranteed a working period of about 1.5 h.

2.4. *Chemometric analysis*

Mean reflectance values of all leaves for each treatment were compared by chemometric multivariate methods to both: each single reference chemical value (named SINGLE) and to all chemical parameters used together (named ALL).

The procedure included the following steps: 1) extraction of raw spectra (X block variables); 2) extraction of measured values (Y block variables); 3) random separation of dataset into two subsets, one for the model (75% of the whole dataset, for the SINGLE and 50% for the ALL) and one for the external validation test (respectively 25% for the SINGLE and 50% for the ALL); for the ALL the dataset was randomised 50 times; 4) application of pre-processing algorithms to both X and Y; 5) application of the chemometric technique PLS (partial least square): modelling and testing; 6) calculation of efficiency parameter of prediction.

To obtain the best prediction test, different X and Y pre-processing techniques were applied, from the simpler (none, mean centre, auto scale, median centre, baseline) to the more specific for spectral data (Savitsky Golay, Multiple Scatter Correction, Orthogonal Signal correction).

The prediction of the nutrients content of leaves was performed using a PLS regression model, using PLS Toolbox in MATLAB V7.0 R14 (The Math Works, Natick, USA). The partial least squares method is a soft-modelling method (Wold, Sjostrom, & Erikssonn, 2001) for constructing predictive models when the factors are many and highly collinear. The model works through a specific algorithm (SIMPLS) on the whole array variables (input variables, X-block) and on the observed values (Y variables) after pre-processing treatments. The model determines the minimum set of the *n* estimation

Table 1 – Descriptive statistical values of elements measured on thirty citrus leaves for each treatment expressed as ppm (parts per million) on dry matter.

	N	P	K	Ca	Mg	Fe	Mn	Zn
Mean	2.532	0.129	0.747	5.096	0.430	100.228	17.262	9.669
St. dev	0.119	0.013	0.347	0.916	0.098	18.086	6.258	2.650
Min	2.240	0.091	0.346	3.180	0.218	66.900	6.690	6.390
Max	2.800	0.224	1.730	10.300	0.927	198.000	42.300	24.800

variables (LV, latent variables) by a recursive process. These variables could be represented in an *n*-dimensional space and they are used by PLS to calculate the best regression matrix between the X and the Y. PLS allows a model to be calculated that was tested on external samples observing its prediction ability. The calibration models were also validated using full cross validation, Venetian blind.

The model includes a calibration phase and a validation phase calculating for both the residual errors (Root mean square error in calibration [RMSEC] and in cross validation [RMSECV]). The prediction ability of the test depends on the number of the LV used in the model and was performed by means of statistical parameters such as RMSE (root mean square error), the SEP (standard error of prevision), the correlation coefficient (*r*) between observed and the predicted values. The values of *r* were taken into consideration to study the correlation between the reference data and the spectral model. Generally, a good model should have high *r*, with low RMSE and SEP values. Therefore, the model was chosen with the minimum number of LV that determines the highest value of correlation between predicted and measured which presents the minimum SEP value. For the analysis that used the ALL chemical values, the pre-processing used on the X and Y block replicated for the 50 cycles of randomisation performed, produced over 50000 models in total. To choose among such a large number of models, the 50 randomisation were averaged and the model with the best performance was selected.

3. Results

Table 1 reports the descriptive statistical data of elemental composition of citrus leaves. The values were in the optimum categories for almost all nutrients, except for K, Mn and Zn that were in the low category.

Table 2 shows the values and results of the PLS prediction of the SINGLE chemical values predicted. The best model ($r = 0.995$) and the test ($r = 0.991$) were obtained for K with a baseline for the X-Block pre-processing algorithm and a mean centre pre-processing for the Y-Block. The prediction ability of such a model was shown to be high with low values for the errors, having SEP = 0.039 and RMSE = 0.039. Finally, the correlation between predicted values and the observed chemical values reported highly significant values with a coefficient of determination (R^2) of 0.9821 (Fig. 2). Also the values and results of PLS prediction of calcium content in leaves were very high. The model gave an *r* value of 0.995 and the test of an *r* value of 0.947.

The results also showed a high efficiency in the estimation of nitrogen leaf content. Both, the model and test PLS prediction showed a high value of *r* (0.945 and 0.909 respectively) (Table 2). The model of this parameter also had low values of SEP (0.039) and RMSE (0.039). Fig. 3 shows the correlation between predicted values and the observed chemical values (N) with a high value of R^2 (0.8265). The lowest values and results of PLS prediction were found for phosphorus ($r = 0.429$). Also, the correlation between measured and predicted values was the lowest ($R^2 = 0.1839$; Fig. 4).

Table 3 shows different results for the ALL elements predicted. Indeed the analysed elements showed *r* values in a range 0.883 (Mg)–0.481 (P) with SEP ranging from 0.01 (P) to 12.418 (Fe). For the construction of the model a large number of LV (19) was used, normalise pre-processing of the X-block and auto scaling for the Y-block.

Table 2 – Results of PLS prediction of the SINGLE chemical parameters.

	Single							
Parameters	N	P	K	Ca	Mg	Fe	Mn	Zn
Model								
N°LV	15	9	15	7	13	10	5	8
Pre-processing X-Block	Baseline	Normalise	Baseline	Osc	Baseline	Gls weighting	Normalise	Baseline
Pre-processing Y-Block	Median centre	Mean centre	Mean centre	Mean centre	Median centre	Median centre	Median centre	Mean centre
RMSEC	0.039	0.003	0.039	0.085	0.020	4.348	3.042	1.099
RMSECV	0.129	0.010	0.144	6.709	0.101	9.675	3.449	2.873
r (observed vs predicted)	0.945	0.915	0.995	0.995	0.982	0.946	0.840	0.905
SEP	0.039	0.004	0.039	0.085	0.020	4.380	3.064	1.107
RMSE	0.039	0.003	0.039	0.085	0.020	4.348	3.042	1.099
Test								
r (observed vs predicted)	0.909	0.429	0.991	0.947	0.944	0.917	0.925	0.889
SEP	0.049	0.018	0.058	0.304	0.048	6.054	1.637	0.972
RMSE	0.051	0.019	0.057	0.614	0.048	6.413	1.683	0.986

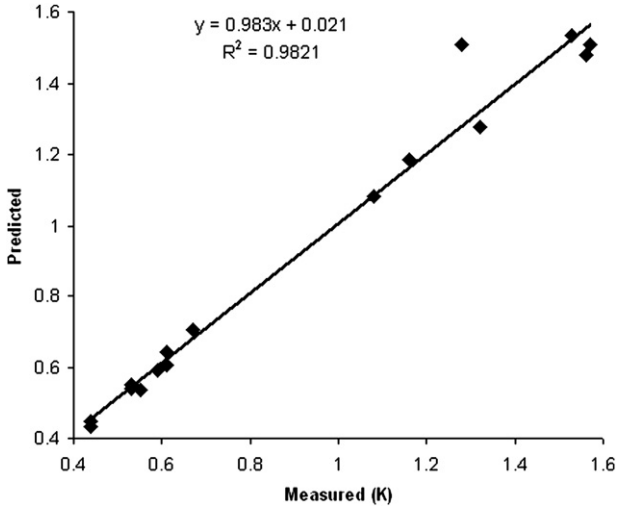

Fig. 2 – Correlation between measured and predicted values of K.

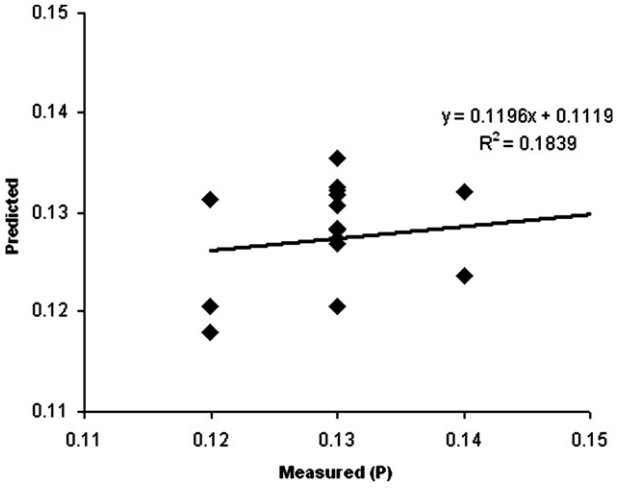

Fig. 4 – Correlation between measured and predicted values of P.

4. Discussion and conclusion

Citrus trees require large quantities of mineral nutrients to attain adequate growth and yield; the needs of these varying with soil fertility and type (Koo *et al.*, 1984). Although the mineral nutrition of citrus trees has been studied intensively, additional information has been frequently published, especially after the introduction of new fertigation technologies and innovative fertilisers (Alva, Fares, & Dou, 2003). The results of this study showed that a system based on a portable spectrophotometer can provide better knowledge of nutritional status of Tarocco orange bearing plants, achieving more detailed and focused information, in a shorter period and over wider areas. The autonomy of the instrument, taking into account the time needed to move from one leaf to the other, allows date acquisitions to perform on about 1200–1300 leaves. Thus, the use of the spectrophotometer, coupled with the multivariate statistical techniques used here gives the possibility to map intensively and precisely large parcel of land, thereby maintaining highly representative samples. This makes the proposed methods suitable for use in precision farming (Alchanatis, Schmilovitch, & Meron, 2005). Furthermore, the possibility of acquiring more detailed information, varying either in space and time, when compared with the standard chemical analysis, should prove to be a useful tool to increase fruit quality and to optimise the use of fertilisers, especially in organic farming systems.

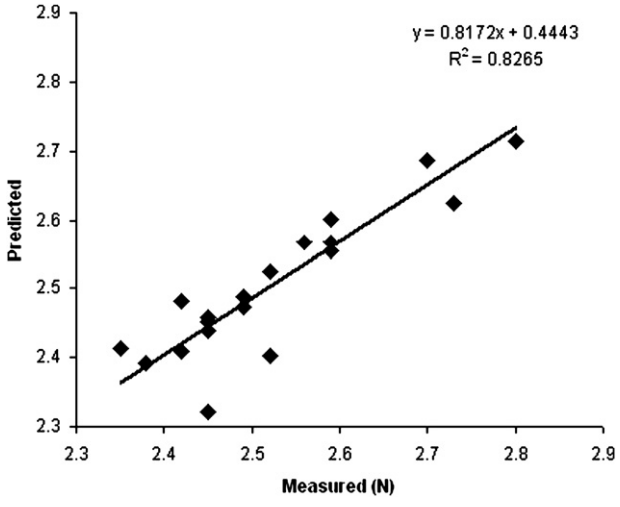

Fig. 3 – Correlation between measured and predicted values of N.

Esposti *et al.* (2003) reported that although the SPAD Chlorophyll Meter proved to be efficient in estimation the N content in leaves, it could not reveal the content of other chemical compounds which the multi-parametric methods proposed here successfully estimated. Moreover, the spectrophotometric technique presented here provided higher levels of correlations for both the model ($r = 0.95$) and the test ($r = 0.91$). Furthermore, such a technique could be able to provide a detailed analytical view of nutrient content, leading to more efficient fertigation planning in citrus orchards. Indeed, satisfactory results were found for the prediction of the SINGLE parameters, often with $r > 0.9$. While some elements scored high values of r, such as N, K, Ca and Mg, others such as P and Zn showed low values probably due to their extremely low concentrations in the leaves, as previously reported in the literature (Embleton, 1973a, 1973b).

Many researchers have successfully used spectral systems to evaluate the N status of different crops (Sui, Wilkerson, Hart, & Howard, 1998; Tumbo, Wagner, & Heinenann, 2002a, 2002b, 2002c). However, even if N can be considered a key nutrient to monitor, the nutritional status of a crop is complex and is given by several parameters. At the beginning of the study numerous standard chemical analysis were carried out, allowing a multiple correlation with the spectral data. This led to the possibility to develop a proper fertilisation strategy to improve the plant nutritional status and reduce the impact on the environment. Moreover the monitoring of different nutrients is essential due to the relationships existing among them. Even if the ALL model (Table 3), showed inferior

Table 3 – Results of PLS prediction of the ALL chemical parameters.

	All							
Parameters	N	P	K	Ca	Mg	Fe	Mn	Zn
Model								
N°LV				19				
Pre–processing X-Block				Normalize				
Pre–processing Y-Block				Autoscale				
RMSEC	0.191							
RMSECV	0.534							
r (observed vs predicted)	0.943	0.957	0.955	0.967	0.976	0.966	0.987	0.950
SEP	0.035	0.003	0.094	0.193	0.019	3.866	0.932	0.669
RMSE	0.035	0.003	0.093	0.191	0.018	3.824	0.922	0.661
Test								
r (observed vs predicted)	0.600	0.481	0.817	0.751	0.772	0.694	0.883	0.506
SEP	0.102	0.010	0.203	0.559	0.062	12.408	2.803	2.255
RMSE	0.103	0.011	0.205	0.566	0.063	12.590	2.849	2.289

performance compared with the SINGLE models (Table 2), it could be an interesting application particularly if rapid measurements are needed.

REFERENCES

Alchanatis, V., Schmilovitch, Z., & Meron, M. (2005). In-field assessment of single leaf nitrogen status by spectral reflectance measurements. *Precision Agriculture, 6*(1), 25–39.

Alva, A. K., Fares, A., & Dou, H. (2003). Managing citrus trees to optimize dry mass and nutrient partitioning. *Journal of Plant Nutrition, 26*, 1541–1559.

Alva, A. K., Paramasivam, S., Graham, W. D., & Wheaton, T. A. (2003). Best nitrogen management practices for citrus production in sandy soils. *Water, Air and Soil Pollution, 143*, 139–154.

Cayuela, J. A. (2008). Vis/NIR soluble solids prediction in intact orange (*Citrus sinensis* L.) cv. Valencia Late by reflectance. *Postharvest Biology and Technology, 47*, 75–80.

Davies, F. S. (1997). *Literature review of research related to nitrogen nutrition, fertilization and groundwater pollution of citrus*. Tallahassee, Fla: Fla. Dep. of Agric. and Consumer Ser., Nitrogen BMP Tech. Group. pp. 70.

Davies, F. S., & Albrigo, L. G. (1994). Citrus. In J. Atherton, & A. Rees (Eds.), *Crop production science in horticulture, Vol. 2*. Wallingford, UK: CAB International.

Embleton, T. W., Coggins, C. W., Jr., & Witney, G. W. (1996). What is most profitable use of citrus leaf analysis? *Proceedings of International Society of Citriculture, 2*, 1261–1264.

Embleton, T. W., Jones, W. W., Labanauskas, C. H., & Reuther, W. (1973a). Leaf analysis as a diagnostic tool and guide to fertilization. In W. Reuther (Ed.), *The citrus industry, Vol. 3* (pp. 183–210). Berkeley, CA, USA: University of California, Div. Agric. Sciences.

Embleton, T. W., Reitz, H. J., & Jones, W. W. (1973b). Citrus fertilization. In W. Reuther (Ed.), *The citrus industry, Vol. 3* (pp. 122–182). Berkeley, CA, USA: University of California, Div. Agric. Sciences.

Esposti, M. D. D., Siqueira, D. L. D., Pereira, P. R. G., Venegas, V. H. A., Salomão, L. C. C., & Machado Filho, J. A. (2003). Assessment of nitrogenized nutrition of citrus rootstocks using chlorophyll concentrations in the leaf. *Journal of Plant Nutrition, 26*(6), 1287–1299.

Ferguson, J. J., Davies, F. S., Tucker, D. P. H., Alva, A. K., & Wheaton, T. A. (1995). Fertilizer guidelines. In D. P. H. Tucker, A. K. Alva, L. K. Jackson, & T. A. Wheaton (Eds.), *Nutrition of Florida citrus trees* (pp. 21–25). Gainesville: University of Florida.

Gomez, A. H., Yong, H., & Pereira, A. G. (2006). Non-destructive measurement of acidity, soluble solids and firmness of *Satsuma mandarin* using Vis/NIR-spectroscopy techniques. *Journal of Food Engineering, 77*, 313–319.

Hanlon, E. A., Obreza, T. A., & Alva, A. K. (1995). In D. P. H. Tucker, A. K. Alva, L. K. Jackson, & T. A. Wheaton (Eds.), *Nutrition of Florida citrus trees* (pp. 13–20). Gainesville: University of Florida.

Hernandez Sanchez, N., Lurol, S., Roger, J. M., & Bellon-Maurel, V. (2003). Robustness of models based on NIR spectra for sugar content prediction in apples. *Journal of Near Infrared Spectroscopy, 11*, 97–107.

Intrigliolo, F., Roccuzzo, G., Lacertosa, G., Rapisarda, P., & Canali, S. (1999). *Agrumi: Modalità di campionamento per terreno, foglie, acque d'irrigazione e frutti; valori analitici di riferimento*. Catania: C.U.E.C.M.

Intrigliolo, F., Tittarelli, F., Roccuzzo, G., & Canali, S. (1998). La fertilizzazione degli agrumi. *L'informatore agrario, 9*, 79–84.

Koo, R. C. J. (1989). Citrus. In D. L. Plucknett, & H. B. Sprague (Eds.), *Detecting mineral nutrient deficiencies in tropical and temperate crops. Westview tropical agriculture series, Vol. 3*.

Koo, R. C. J., Anderson, C. A., Stewart, I., Tucker, D. P., Calvert, D. V., & Wutscher, H. K. (1984). Recommended fertilizers and nutritional sprays for citrus. *Florida Agriculture Experiment Station Bulletin, 536D*. USA.

Lee, W., Searcy, S. W., & Kataoka, T. (1999). *Assessing nitrogen stress in corn varieties of varying color*. ASAE Paper No. 993034. St. Joseph, MI, USA: ASAE.

Legaz-Parades, F., & Primo Millo, E. (2000). Criterios para la fertilización de los cítricos en riego localizado por goteo. In J. F. Giner (Ed.), *Curso de fertirrigación de cítricos* (pp. 137–155). Phytoma: Universitat Politécnica de Valencia - Generalitat Valenciana.

McGlone, V. A., Fraser, D. G., Jordan, R. B., & Kunnemeyer, R. (2003). Internal quality assessment of mandarin fruit by Vis/NIR spectroscopy. *Journal of Near Infrared Spectroscopy, 11*, 323–332.

Miller, W. M., & Zude-Sasse, M. (2004). NIR-based sensing to measure soluble solids content of Florida citrus. *Applied Engineering in Agriculture, 20*, 321–327.

Obreza, T. A., Alva, A. K., Hanlon, E. A., & Rouse, R. E. (1992). Citrus grove leaf tissue and soil testing: sampling, analysis, and

interpretation. *University of Florida, Cooperative Extension Service Bulletin, SL, 115*, 1–4.

Piekielek, W. P., Fox, R. H., Toth, J. D., & Macneal, K. E. (1995). Use of a chlorophyll meter at the early dent stage of corn to evaluate nitrogen sufficiency. *Agronomy Journal, 87*, 403–408.

Read, J. J., Tarpley, L., McKinion, J. M., & Reddy, K. R. (2002). Narrow-waveband reflectance ratios for remote estimation of nitrogen status in cotton. *Journal of Environmental Quality, 31*, 1442–1452.

Saranwong, S., Sornsrivichai, J., & Kawano, S. (2003a). On-tree evaluation of harvesting quality of mango fruit using a hand-held NIR instrument. *Journal of Near Infrared Spectroscopy, 11*, 283–293.

Saranwong, S., Sornsrivichai, J., & Kawano, S. (2003b). Performance of a portable near infrared instrument for Brix value determination of intact mango fruit. *Journal of Near Infrared Spectroscopy, 11*, 175–181.

Steuer, B., Schulz, H., & Lager, E. (2001). Classification and analysis of citrus oils by NIR spectroscopy. *Food Chemistry, 72*, 113–117.

Sui, R., Wilkerson, J. B., Hart, W. E., & Howard, D. D. (1998). *Integration of neural networks with a spectral reflectance sensor to detect nitrogen deficiency in cotton*. ASAE Paper No. 983104. St. Joseph, MI, USA: ASAE.

Temma, T., Hanamatsu, K., & Shinoki, F. (2002). Development of a portable near infrared sugar-measuring instrument. *Journal of Near Infrared Spectroscopy, 10*, 77–83.

Tumbo, S. D., Wagner, D. G., & Heinenann, P. H. (2002a). Hyperspectral-based neural network for predicting chlorophyll status in corn. *Transactions of the ASAE, 45*, 825–832.

Tumbo, S. D., Wagner, D. G., & Heinenann, P. H. (2002b). On-the-go sensing of chlorophyll status in corn. *Transactions of the ASAE, 45*, 1207–1215.

Tumbo, S. D., Wagner, D. G., & Heinenann, P. H. (2002c). Hyperspectral characteristics of corn plants under different chlorophyll levels. *Transactions of the ASAE, 45*, 815–823.

Ventura, M., De Jager, A., De Putter, F. H., & Roelofs, F. F. (1998). Non-destructive determination of soluble solids in apple fruit by near infrared spectroscopy (NIRS). *Postharvest Biology and Technology, 14*, 21–27.

Walsh, K. B., Guthrie, J. A., & Burney, J. W. (2000). Application of commercially available, low-cost, miniaturised NIR spectrometers to the assessment of the sugar content of intact fruit. *Australian Journal of Plant Physiology, 27*, 1175–1186.

Wold, S., Sjostrom, M., & Erikssonn, L. (2001). PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory System, 58*, 109–130.

Zude, M., Herold, B., Roger, J. M., Bellon-Maurel, V., & Landahl, S. (2006). Non-destructive tests on the prediction of apple fruit flesh firmness and soluble solids content on tree and in shelf life. *Journal of Food Engineering, 77*, 254–260.