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Ph.D. COURSE IN

ENVIRONMENTAL SCIENCES - XXIV Cycle

OPTOELECTRONIC FOR AGRO-ENVIRONMENTAL SUSTAINABILITY: FROM IN-FIELD SOIL PREPARATION TO PRE- AND POST-HARVEST APPLICATIONS

ING-INF/06

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Abstract

In the developed countries, the environment is mainly used as agricultural land. Nowadays, the increasing use of precision farming practices led to review the information on the agro-environmental production parameters along all the agricultural chain. In this Ph.D. research three parameters have been monitored: soil water content, crop nutritional status and fruit quality. The aim was to evaluate potential applications of rapid and non-destructive optoelectronic techniques to reduce environmental impact in in-field soil preparation, pre- and post-harvest phases. Regarding the in-field soil preparation the scope was to investigate the suitability of active infrared thermography and thermometry, combined with multivariate statistical analysis, for a rapid laboratory and in-field detection of soil water content. Concerning the pre-harvest two case studies were conducted. The first proposed the use of visible-near infrared portable spectrophotometer to evaluate citrus crop nutritional status through foliar analysis. The second evaluated the possibility and the accuracy of the same system, in comparison with conventional analyses, in estimating tomato leaf nitrogen concentration. In the post-harvest case study was developed a non-destructive estimation of citrus fruit internal quality parameters using the mentioned portable spectrophotometer. This Ph.D. activity could provide the basis for future applications such as monitoring and stabilising crop nutritional levels, assessing rapidly soil water content and fruit internal quality and reducing environmental impact. These case studies were finally published in international peer-reviewed journals with impact factors.

Riassunto

Nei paesi sviluppati, l'ambiente è principalmente utilizzato come terreno agricolo. Ad oggi, l'aumento delle pratiche di agricoltura di precisione ha portato a riesaminare le informazioni riguardanti i parametri di produzione agro-ambientale in tutte le fasi della catena agricola. In questo dottorato di ricerca, tre di questi parametri sono stati monitorati: il contenuto d'acqua del suolo, lo stato nutrizionale delle colture e la qualità dei frutti. L'obiettivo era quello di valutare le potenziali applicazioni delle rapide e non distruttive tecniche optoelettroniche per ridurre l'impatto ambientale nelle fasi di preparazione del terreno direttamente in campo ed in quelle di pre- e post-raccolta. Riguardo alla fase di preparazione del suolo, lo scopo era quello di valutare l'idoneità della termografia e della termometria attiva ad infrarossi, combinata con l'analisi statistica multivariata, per una rapida individuazione del contenuto idrico del suolo sia in laboratorio che in campo. Nella fase di pre-raccolta due casi di studio sono stati condotti. Il primo ha proposto l'utilizzo di uno spettrofotometro portatile del visibile e vicino infrarosso per valutare lo stato nutrizionale di colture di agrumi attraverso l'analisi fogliare. Il secondo ha valutato la possibilità e l'accuratezza dello stesso sistema, a paragone con le tecniche convenzionali, nello stimare la concentrazione fogliare di azoto in piante di pomodoro. Nel caso di studio della fase di post-raccolta è stato sviluppato un sistema non distruttivo per stimare dei parametri di qualità interna di agrumi usando il già menzionato spettrofotometro portatile. Questa attività di Ph.D. potrebbe fornire le basi per future applicazioni come il monitoraggio e la stabilizzazione dei livelli nutrizionali delle colture, la rapida valutazione del contenuto d'acqua del suolo e della qualità interna dei frutti e la riduzione dell'impatto ambientale. In fine, questi casi di studio sono stati pubblicati su riviste internazionali peer-reviewed con impact factor.

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1. Aim and Objectives

In the developed countries, the environment is mainly used as agricultural land. Agriculture shapes half of European landscapes by providing large seasonal, functional and structural variations impacting on key components of the biosphere (soil-water-vegetation-air). Moreover, it plays an essential role in maintaining the environment in a healthy state even if the links between the natural environment and farming practices are complex. In fact, agriculture and environment exercise a profound influence over each other. On the one hand, farming has contributed over the centuries to creating and maintaining a variety of valuable semi-natural habitats which now host a relevant part of European biodiversity and often represent an important cultural heritage. On the other hand, agricultural practices can also exert an adverse impact on natural resources. Pollution of soil, water and air, fragmentation of habitats and loss of wildlife can represent the result of inappropriate agricultural practices and land use (http://agrienv.jrc.ec.europa.eu/index.htm).

Generally, the term “sustainable”, or “eco-compatible”, agriculture refers to a system capable of maintaining its productivity and usefulness to society over the long run. As reported by van der Werf and Petit (2002), farmers adjust their production practices (e.g., tillage operations, sowing, and fertilisation) in order to optimally combine inputs based on natural capital (soil, solar energy, rain, fossil energy) and inputs from human-made capital (fertilisers, seeds, pesticides) yielding desired outputs (products) and undesired emissions to the environment. As a consequence, in these last years, the demand of new equipments, and/or devices, able to realize innovative analytical and quality control strategies, strongly increased. Thanks to these innovations it is possible to fulfil specific targets related to a more strict monitoring of production and products in order to realize a precise standardization, both in qualitative terms and marketable goods characteristics, reducing, at the same time, the environmental impact (Bonifazi et al., 2004). Systems and devices such as for example Geographical Positioning System (GPS) and sensors, are of a foremost importance for an efficient data collection required in agricultural practices and in others activities such as precision agriculture that represents a kind of agriculture increasing the number of correct decisions per unit area of land per unit time with associated net benefits (McBratney et al., 2005). For example, the precision agriculture allows producers to perform a tailored use of fertilizers in lieu of an arbitrary application at uniform rates over large areas. For these reasons, the concepts of precision agriculture and sustainability are inextricably linked (Bongiovanni & Lowenberg-Deboer, 2004). The concept of precision agriculture refers to precision farming and to precision production that are strictly correlated. The goal of precision farming is to improve the profits of farmers and harvest yields with a concomitant reduction of negative impacts on the environment mainly due to a misuse of agrochemicals. All these practices determine the level of the agricultural production (precision production).

In these terms there is an increasing need to review information on the agro-environmental production parameters along all the agricultural chain "*from the cradle to the grave*" represented for example by: yield distributions, soil fertility (N, P, K, Ca, Mg, C, Fe, Mn, Zn, and Cu content), soil density, soil mechanical strength, soil water content, crop density, crop height, crop nutritional status (N, P, K, Ca, Mg, C, Fe, Mn, Zn, and Cu), crop water stress, weed infestation, insect infestation, tillage practice, crop rotation, fertilizer application, irrigation pattern, fruit quality, etc.

In this Ph.D. research three of these agro-environmental production parameters have been monitored:

1. soil water content;
2. crop nutritional status;
3. fruit quality.

The general aim of this Ph.D. activity was to evaluate potential applications of non-invasive, rapid and non-destructive optoelectronic techniques to reduce environmental impact in the in-field soil preparation, pre- and post-harvest phases (Fig. 1.1).

Regarding the in-field soil preparation a rapid soil water content detection by active infrared thermal methods was developed. The aim was to investigate the suitability of active infrared thermography and thermometry, combined with multivariate statistical analysis, for a rapid laboratory and in-field detection of soil water content. These techniques allowed fast soil water content measurements helpful in both agricultural and environmental fields (Fig. 1.1).

Concerning the pre-harvest phase, two case studies were conducted. The first proposed the use of visible-near infrared portable spectrophotometer to evaluate citrus crop nutritional status represented by various macroelements (i.e., N, P, K, Ca, Mg, Fe, Zn and Mn) through foliar analysis. The second evaluated the possibility and the accuracy of the same system, in comparison with conventional analyses (i.e., chemical standard analyses, chlorophyll meter readings and $N\text{-}NO_3$ concentration in petiole sap), in estimating tomato leaf nitrogen concentration (Fig. 1.1).

For the post-harvest phase one case study was developed in order to assess possibility and limits of a non-destructive estimation of citrus fruit internal quality parameters (i.e., total soluble solids and titratable acidity) presenting thick peel by using the mentioned portable spectrophotometer (Fig. 1.1).

All these case studies were published in international peer-reviewed journals with impact factors.

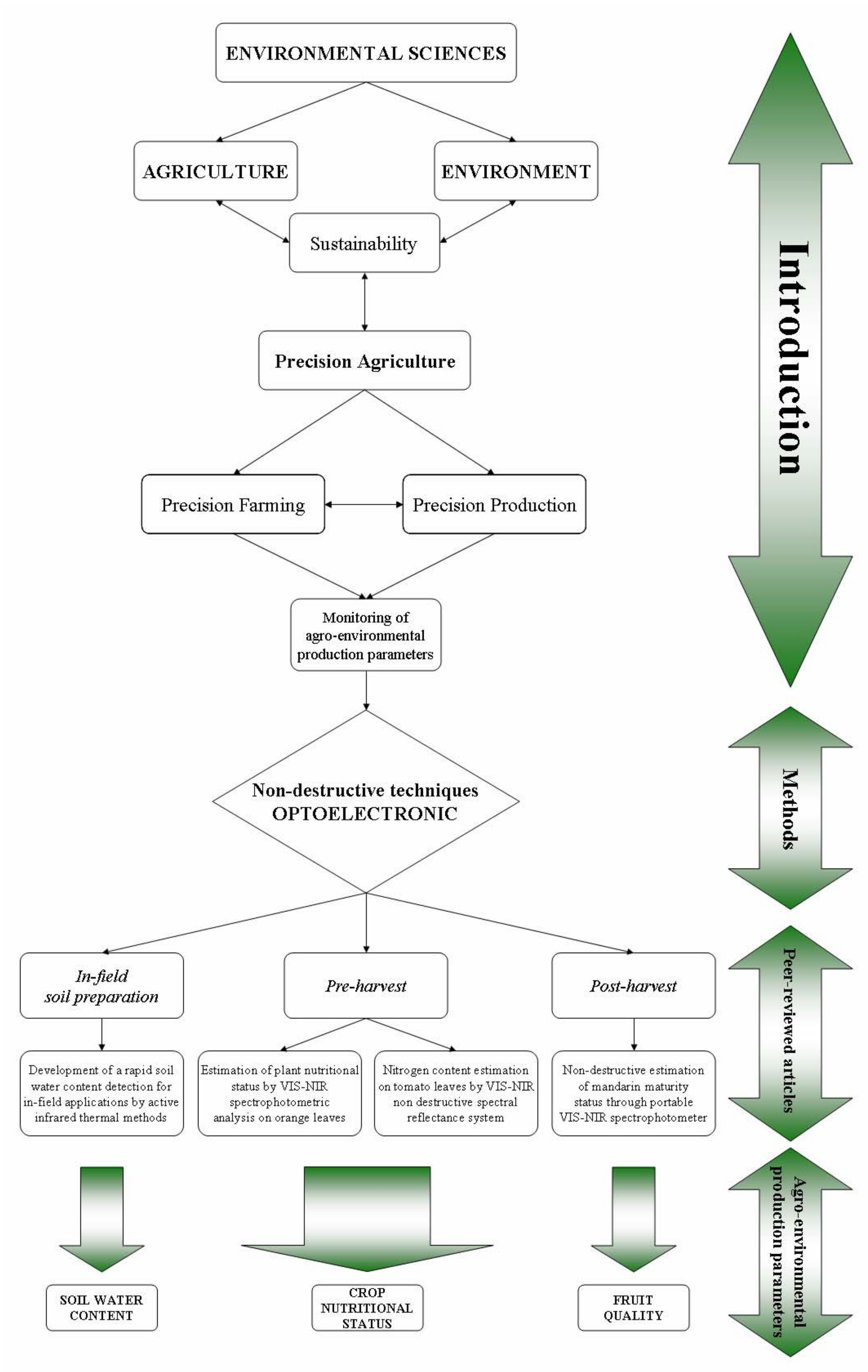

Figure 1.1: Flow chart of the research activities of this Ph.D. Thesis.

2. Introduction

2.1 Agro-environmental sustainability

Over the past two decades, increasing awareness of the ecosystems fragility and increasing concern for the state of the environment has focused attention on the effect of agricultural activities on crop, soil and water quality. In many countries, research studies and extension programs have been instituted by governments and universities to encourage farmers to adopt conservation practices such as forage rotations and cover crops, reduced tillage, maintenance of crop residues and reduced fallowing. As a result, considerable adjustment of land use and production activities has occurred.

Recently, as reported by Huffman et al. (2000), as the concept of sustainable production becomes more accepted, decision-makers are striving to develop environmentally friendly policies and programs. The need for quantitative information on the extent, degree and regional variability of the environmental impact of agricultural production practices, and on the adoption of conservation practices, has become immediate.

The implications of this in terms of data, especially in the complex, interrelated and broad spatial context of ecosystems is massive (Huffman et al., 2000). In the absence of complete information about these relationships, the concept of rapid in-field applications serving as proxies for a wider variety of parameters has been proposed.

Agriculture today must balance a wide array of demands and environmental challenges that are continually evolving in their nature and complexity. A major challenge is achieving long term environmental sustainability of production (McRae, 2000). As reported by Pacini et al. (2003) agricultural researchers widely recognise the importance of sustainable agricultural production. The word sustainable is derived from the Latin *sustinere* meaning to keep in existence, implying permanence or long-term support. In the context of agricultural production, Ikerd (1993) defines a sustainable agriculture as capable of maintaining its productivity and usefulness to society over the long run. Moreover, it must be environmentally-sound, resource-conserving, economically viable and socially supportive, commercially competitive, and environmentally sound. The complex nature of the interrelationships between agricultural production and the natural environment means that we far from know which methods and systems in different locations will lead to sustainability (Youngberg & Harwood, 1989). As reported by Rigby & Cáceres (2001) this seems to be a crucial issue in the debate, and leads one to ask how long should an agro-system behave sustainably to be considered sustainable, and how should sustainability could be assessed.

Farmers adjust their production practices (e.g. tillage operations, sowing, and fertilisation) in order to optimally combine inputs based on natural capital (soil, solar energy, rain, fossil energy) and inputs from human-made capital (fertilisers, seeds, pesticides) yielding desired outputs (products) and undesired emissions to the environment (van der Werf & Petit, 2002).

Crop nutrients and pesticides are added to agroecosystems to improve crop production. When not used wisely, these amendments can reduce the quality of soil, water, and air and can affect biodiversity (McRae, 2000). Generally, fertilizer application methods that reduce nutrient losses were quite prevalent, although room for improvement exists. Applying nitrogen in excess of crop needs reflects inefficient nutrient management, incurs unnecessary costs, and poses a threat to water quality. Movement of nitrogen into the atmosphere as ammonia and nitrous oxide contributes to poor air quality and potentially to global warming. As agriculture continues to move to larger and more intensive operations, sound input management practices will be critical for both environmental protection and farm profitability. In most cases, improving input management goes hand in hand with enhancing farm profitability (McRae, 2000).

The intensification of production methods caused increasing environmental pollution and consequently, the limitation of the environmental impacts to acceptable level became increasingly important in agricultural research (van der Werf & Petit, 2002). This generated a large amount of research concerning the impacts of agriculture on the environment (e.g. Wauchope, 1978; Ryden et al., 1984). As reported by Yang et al. (2003) modern conventional agriculture is heavily dependent on chemical inputs to promote high crop yields. Moreover, conventional agriculture tends to ignore spatial variability within fields. Agrochemicals have been applied uniformly over production areas, resulting in excesses in some locations and insufficient intervention at others. The over-application of these agrochemicals has become one of the main sources of pollution (Mannion, 1995; Paice et al., 1996). The extra agrochemicals remain in the soil, leach into the ground water and/or drain into surface water bodies (Smith et al., 1995; Paice et al., 1996). Pollution and contamination from agrochemicals in soil, ground water and runoff can cause various illnesses, including disorders of the nervous system (Peralta et al., 1994; Mannion, 1995). Pesticides also reduce the population of some natural predators of harmful pests, thus occasionally resulting in an increase rather than a decrease in the population of the target pest, and causing increased losses (Mannion, 1995). Consequently, improved management practices that reduce the over-application of agrochemicals have become an important focus of research. One approach to reducing chemical inputs is precision farming also called site-specific farming. This allows farmers to manage what happens in the field at a very fine spatial resolution and can thus increase the efficiency of the farming enterprise (Ascheman, 1993; Blackmore, 1994; Paice et al., 1996). It enables the application of agrochemical inputs and the adoption of other farming practices that match the inherent variability in a field (Paice et al., 1996). Although many of the technologies used in precision farming require further research and development, it has already yielded considerable benefits by lowering costs, increasing production and improving the environmental condition (Yang et al., 2003).

Work on impact assessment raises the issue of which are the key aspects of a system's performance that should be measured, that is, what are the key aspects of agricultural sustainability (Rigby & Cáceres, 2001). The level of production of agro-ecosystems mainly depends on natural and human-made capital inputs. Herdt & Steiner (1995) indicate that it is hard to know whether current agro-ecosystems are sustainable in the sense of remaining productive in the long run.

Environmental impact of agriculture depends to a large extent on farmer production practices. The link however is indirect, as emissions to the environment depend on the state of the farming system, which in turn depends on farmer production practices but also on random factors such as rainfall and temperature (van der Werf & Petit, 2002). Modern conventional agriculture is heavily dependent on chemical inputs to promote high crop yields. Conventional agriculture has tended to ignore spatial variability within fields. Agrochemicals have been applied uniformly over production areas, resulting in excesses in some locations and insufficient intervention at others (Yang et al., 2003).

In order to effectively evaluate environmental impact, rapid, non-destructive and non-invasive evaluation methods should take into account a range of objectives covering both local and global effects. The number of objectives should be sufficiently large to avoid the inadvertent creation of new problems, and as small as possible to maintain feasibility, they should not be redundant. The procedure used for the selection of objectives should be stated. Numerous attempts to develop on-the-go sensor techniques to analyse agro-environmental properties and to increase the efficiency of precision farming (Pierce & Nowak, 1999) have been developed in the last years. Moreover, to an appropriate management of agricultural practices, methods increasing the density of soil characterisation at a relatively low cost are required (Sonka et al. 1997).

Engineering knowledge and expertise make important contributions to all portions of the system. Systems and devices such as for example Geographical Positioning System (GPS), sensors and sample collection equipment, are essential for the efficient collection data required in agricultural practices and in others activities such as precision farming (Sudduth, 1999). These developments have led to a variety of methods for the evaluation of the environmental impacts of agriculture. The development of such tools is considered by many authors as a condition for the implementation of a sustainable agriculture (e.g. Hansen, 1996). These methods take into account a number of environmental issues of concern (e.g. soil erosion, emission of greenhouse gasses, water quality).

2.2 Precision Agriculture

Precision agriculture has generated a very high profile in the agricultural industry over the last decade of the second millennium. With the advent of the satellite-based GPS, farmers gained the potential to take account of spatial variability. The topic has been technology-driven and so many of the engineering developments are in place, with understanding of the biological processes on a localized scale lagging behind. Precision agriculture, as a crop management concept, can meet much of the increasing environmental, economic, market and public pressures on arable agriculture (Stafford, 2000) and it represents a kind of agriculture that increases the number of (correct) decisions per unit area of land per unit time with associated net benefits (McBratney et al., 2005). It should be considered as a philosophical shift in the management of variability within agricultural industries. It must be aimed at improving profitability and or environmental impact both short and long term (Whelan & McBratney, 2000).

Some of the competitive advantage of precision agriculture will come from the in-field separation of product into quality classes. Economic benefits will come especially if there are non-linearities in the payment of quality premiums. Quality criteria are particularly important for the high-value crops. A secondary benefit of this approach is the mapping of quality characteristics to improve agronomic management for optimising the quantity/quality. A lot of work is needed on developing quality criteria and sensor systems in a product-chain approach which will make it feasible to interact effectively with customers (McBratney et al., 2005). This scenario calls for the introduction of modern technologies to improve crop yield, provide information to enable better in-field management decisions, reduce chemical and fertilizer costs through more efficient application, permit more accurate farm records, increase profit margin and reduce pollution (Sparovek & Schnug, 2001).

Generally, precision agriculture requires a method of gathering information about the spatial variability of soil that reduces the need for expensive and intensive sampling (McBratney & Pringle, 1999). It potentially provides producers improved tools to manage those inputs that must be brought to the farm. Instead of indiscriminately applying fertilizer or pesticides at uniform rates over large areas, it allows producers to better target applications. The hope of PA is that its use will be less disruptive of natural systems than uniform application of physical inputs has been (Bongiovanni & Lowenberg-Deboer, 2004).

The concepts of precision agriculture and sustainability are inextricably linked as reported by Bongiovanni & Lowenberg-Deboer (2004). From the first time a global positioning system was used on agricultural equipment the potential for environmental benefits has been discussed. Intuitively, applying fertilizers and pesticides only where and when they are needed, should reduce environmental loading.

In this context it is possible to distinguish the precision agriculture into two main points: farming and production.

2.2.1 Precision farming

Precision farming is a farming management concept based on observing and responding to intra-field variations. The concept of precision agriculture first emerged in the United States in the early 1980s. Towards the end of the 1980s, this technique was used to derive the first input recommendation maps for fertilizers and pH corrections (http://en.wikipedia.org/wiki/Precision_agriculture). The goal of precision farming is to improve farmers' profits and harvest yields while reducing the negative impacts of farming on the environment that come from over-application of chemicals.

As reported by Sudduth (1999) the precision farming approach to crop production may be viewed as a four-step process (Fig. 2.2.1.1).

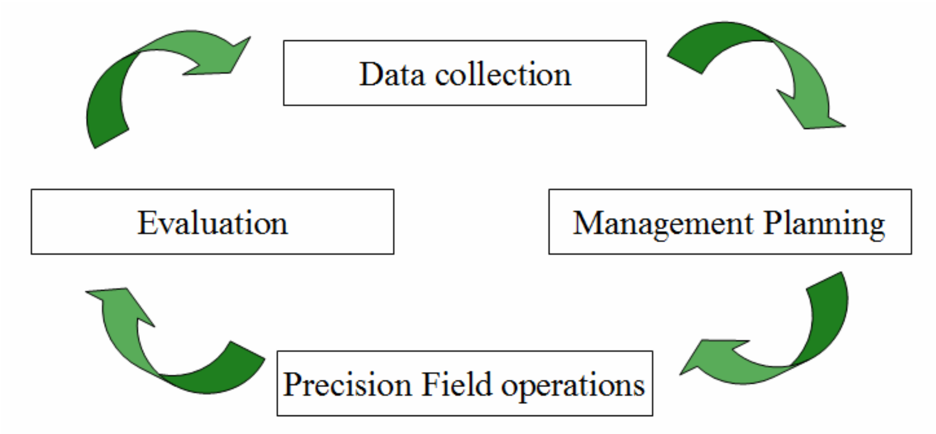

Figure 2.2.1.1: The cyclic scenery of the precision farming approach (adapted from Sudduth, 1999).

An initial step in this process is spatial measurement of those factors that limit or otherwise affect crop production. These variability data are then used to develop a management plan for the variable application of inputs such as fertilizers and herbicides. Inputs are applied in precision field operations. Finally, the effectiveness of the precision farming system is evaluated with respect to economics and environmental impacts. This evaluation becomes a part of the data collection process for the next cropping season. Multiple iterations through the cycle allow for refinement of the precision management plan in succeeding seasons.

Some of the applications of remote sensing in precision farming are shown in Figure 2.2.1.2 with three false-colour images. The top image shows the colour variations determined by crop density, where dark blues and greens indicate lush vegetation and reds show areas of bare soil. The middle image is a map of water deficit, derived from reflectance and temperature measurements. Greens and blues indicate wet soil and reds are dry soil. The bottom image shows where crops are under serious stress, as is particularly the case in Fields 120 and 119 (indicated by red and yellow pixels). These fields were due to be irrigated the following day. (http://earthobservatory.nasa.gov/IOTD/view.php?id=1139).

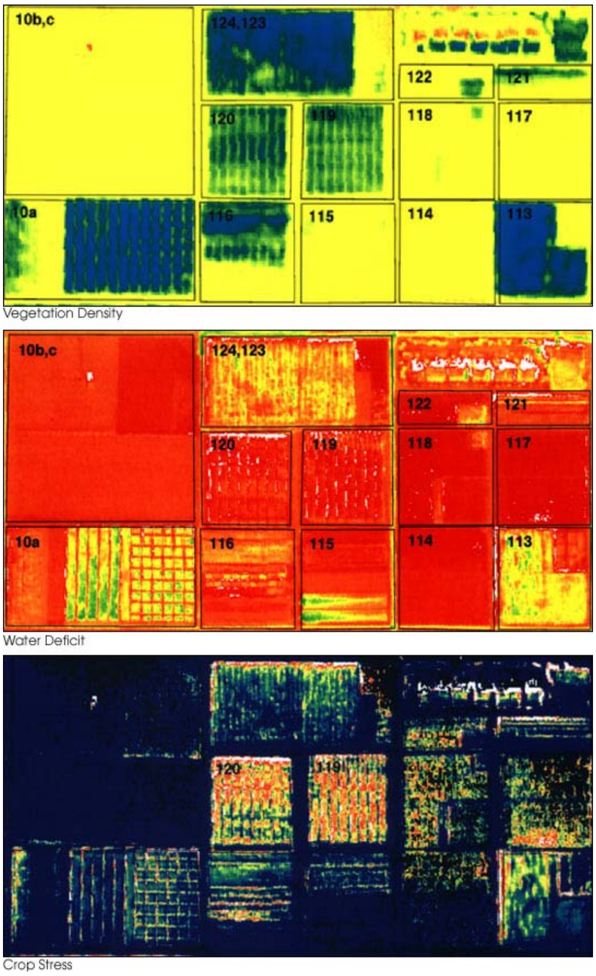

Figure 2.2.1.2: Three false-colour images to demonstrate some of the applications of remote sensing in precision farming: the top image shows the colour variations determined by crop density; the middle image is a map of water deficit, derived from reflectance and temperature measurements and the bottom image shows where crops are under serious stress (from http://earthobservatory.nasa.gov/IOTD/view.php?id=1139).

As reported by Batte & Arnholt (2003) the precision farming has the potential to help farmers improve input allocation decisions, thereby lowering production costs or increasing outputs, and, potentially, increasing profits. However, with the enlargement of fields and intensive mechanization, it has become increasingly more difficult to take account of within-field variability without a revolutionary development in technologies (Stafford, 2000). In this context precision farming is conceptualized by a system approach to re-organize the total system of agriculture towards a low-input, high-efficiency and sustainable agriculture (Shibusawa, 1998). Zhang et al. (2002) indicates that this new approach mainly benefits from the emergence and convergence of several technologies such as: GPS, geographic information system (GIS), miniaturized computer components, automatic control, in-field and remote sensing, mobile computing, advanced information processing, and telecommunications. Agricultural industry is now capable of gathering more comprehensive data on production variability in both space and time. The desire to respond to such variability on a fine-scale has become the goal of precision farming (Whelan et al., 1997).

At the close of the 20th century, precision farming has evolved into a current research topic all over the world. Today, most agricultural disciplines participate in this effort, encouraging progress in agricultural technology, which originally supplied the impetus through the integration of information technology into tractors, machines and implements. Moreover, the term is almost always associated with the site-specific fertilisation and generally, farmers using this new technology to decrease in fertiliser required for the same yield and or higher yields with the use of the same amounts of fertiliser. Precision farming today aims at heterogeneities within a crop, attempting to take into account the requirements and environment. Thus, this agricultural system is geared toward large-scale farming, increasing its existing production advantages (Auernhammer, 2001).

2.2.2 Precision production

The precision production is measured as the ratio of agricultural outputs to agricultural inputs. These output values may be compared to many different types of inputs such as labour and land (yield). Although the use of some inputs like fertilizer and machinery increased, these increases were more than offset by reductions in cropland and especially the amount of labor employed in agriculture (Fuglie et al., 2007).

Agricultural practices determine the level of food production and, to a great extent, the state of the global environment (Tilman et al., 2002). Agriculturalists are the chief managers of terrestrial useable lands of which about half of these is already in pastoral or intensive agriculture (Tilman et al., 2001). Adapting production inputs site-specifically within a field and individually for each animal allows better use of resources to maintain the quality of the environment while improving the sustainability of the food supply (Gebbers & Adamchuk, 2010).

In these terms, the precision production could provide a tool to supervise food production chain managing both the quantity and quality of agricultural produce. In the past, it was difficult for farmers to correlate production techniques and crop yields with land variability. This limited their ability to develop the most effective soil/plant treatment strategies that could have enhanced their production. Today, more precise application of pesticides, herbicides, and fertilizers, and better control of the dispersion of those chemicals are possible through precision agriculture, thus reducing expenses, producing a higher yield, and creating a more environmentally friendly farm (http://www.gps.gov/applications/agriculture/).

In these last years, the demand of new equipments to realize innovative quality control strategies and to review information on the agro-environmental production parameters strongly increased.

2.3 Agro-environmental production parameters

As reported by Zhang et al. (2002) the variability influencing agricultural production can be categorized into six groups and for each group some of agro-environmental production parameters can be considered:

1. yield variability: historical and present yield distributions;
2. field variability: field topography elevation, slope, aspect, and terrace, proximity to field boundary and streams, etc.;
3. soil variability: soil fertility representing by N, P, K, Ca, Mg, C, Fe, Mn, Zn, and Cu content; soil fertility as provided by manure; soil physical properties-texture, density, mechanical strength, moisture content, and electric conductivity; soil chemical properties/pH, organic matter and salinity; soil plant-available water-holding capacity and hydraulic conductivity; and soil depth;
4. crop variability: crop density; crop height; crop nutrient stress for N, P, K, Ca, Mg, C, Fe, Mn, Zn, and Cu; crop water stress; crop biophysical properties/leaf-area index (LAI), intercepted photosynthetically active radiation, and biomass; crop leaf chlorophyll content; and crop grain quality;
5. variability in anomalous factors: weed infestation; insect infestation; nematode infestation; disease infestation; wind damage, and hay damage;
6. management variability: tillage practice; crop hybrid; crop seeding rate; crop rotation; fertilizer application; pesticide application; and irrigation pattern.

Generally among these variability types, yield variability is often considered the ultimate dependent variable, whereas most other variability types are treated as independent variables. The most extensively studied independent variable to date has been soil nitrogen fertility level. In fact, most variable-rate technologies for chemical applications have been developed on nitrogen-fertilizer applicators (Zhang et al., 2002).

Many types of variability are both spatial and temporal in nature. Weed infestation serves as an example. Spatial weed-patch patterns may change during the crop-growing season. Variability in climate parameters is mostly temporal in nature. However, intensive precipitation monitoring across fields is also important to assisting decision making for fertilizer applications (O'Neal et al., 2000).

Zhang et al. (2002) reported as two kind of approaches can be achieved to manage the variability: the map-based approach and the sensor-based approach. Moreover, control decisions for variable rate application can be implemented either on-line or off-line. In the on-line or sensor-based approach, the controlled equipment incorporates onboard sensors and the sensor data are used immediately for automatic control. In the off-line or map-based approach, data are collected and stored in one operation, and the controlled equipment uses the information in a separate field operation. The map-based approach allows more flexibility in data manipulation and pre-processing but requires that the location of equipment in the field be precisely defined, as with GPS. Most systems currently available are map-based, but more on-line systems will likely become available as real-time sensing technologies become more mature. Hybrid systems which rely on a combination of both mapped and real-time data may also come into more widespread use (Sudduth, 1999).

2.3.1 Map-based managing of agro-environmental production parameters

The map-based approach requires the following procedure: grid sampling a field, performing laboratory analyzes of soil samples, generating a site-specific map, and, finally, using this map to control a variable-rate applicator. A positioning system, such as a GPS, is usually required for this approach. Site-specific applications of agricultural inputs can be implemented by dividing a field into smaller management zones that are more homogeneous in properties of interest than the field as a whole. A management zone is defined by Doerge (1998) as a portion of a field that expresses a homogeneous combination of yield-limiting factors for which a single rate of a specific crop input is appropriate. A management zone also can be delineated by more than one specific crop inputs. In this case, a single rate is applied for each of the specific inputs within a zone. The number of distinct management zones within a field is a function of the natural variability within the field, the size of the field, and certain management factors. The minimum size of a zone is limited by the ability of the farmer to differentially manage regions within a field (Zhang et al., 2002). If a GPS is involved to control the application or to guide the implement, there seems no reason for restrictions on the shape of the zone. However, in reality, the pattern in which the application equipment traverses the field should be considered when delineating the management zones (Kvien & Pocknee, 2000).

2.3.2 Sensor-based managing of agro-environmental production parameters

The sensor-based approach, on the other hand, measures the desired properties, such as soil and plant properties, using real-time sensors in an "on-the-go" fashion and controls variable-rate applicator based on the measurements. For this approach, a positioning device is not always needed. Sudduth (1999) reports that it is especially important to develop and implement sensor technology for those parameters, such as soil nitrate and soil moisture, which can change rapidly (both spatially and temporally) and must be measured in real-time or near real-time to be useful for input control. Sensors will allow the collection of data on a much finer spatial resolution than is currently feasible with manual and/or laboratory methods, to more accurately characterize within-field variability.

As reported by Auernhammer (2001) historical data are not enough if high yields are based on nitrogen fertilisation, livestock breeding ensures adequate nutrient supply with P and K, and the weather is subject to great changes. For such conditions, the sensor approach monitors actual growth conditions over time. To do this, plant chlorophyll reflectance is a very useful measurement because it correlates closely with nitrogen content of the plant and with resulting plant mass. At the same time, reflectance in the near infrared area is distinguishable easily from surrounding plant matter and soil. In combination with type-specific growth functions for individual plants, growth deficits may be detected and remedied by real-time application of nitrogen-fertilisers.

With respect to fertilisation, the problems lie in plant-specific application of nitrogen taking into account the soil water available to the plant. Several improvements are needed.

Some examples of sensor-based managing are represented by the optoelectronic techniques as the near infrared (NIR) soil sensor measuring soil spectral reflectance to predict soil organic matter and moisture contents of surface and subsurface soils (Hummel et al., 2001). The on-line, real-time soil spectrophotometer measures soil spectral reflectance in the visible and NIR wavebands at a ground speed of 3.6 km/h. Field tests demonstrated linear relationships between reflectance at certain wavelengths and various soil properties, including soil organic matter and moisture content (Shibusawa et al., 2000). Another example is reported by Thai et al. (1999): in their study a field spectral-imaging system with a liquid crystal tunable filter in peanut and cotton fields was used. In the work of Stafford & Bolam, (1998) a near-ground scanning radiometer was mounted on a tractor to map vegetative-indices. Sudduth et al. (2000) designed an electromechanical sensor to count corn plants. Cotton plant height was measured using mechanical fingers and infrared light beams (Searcy & Beck, 2000). An infrared thermometer was used to measure canopy temperature to control irrigation events (Evans et al., 2000). A microwave sensor, and a NIR sensor were tested to measure moisture content of forage

(Marcotte et al., 1999). An on-line, real-time spectrophotometer developed by Anom et al. (2000) was used to map plant water, nutrient, disease, and salinity stresses. Michels et al. (2000) designed an infrared plant-temperature transducer to sense plant temperature changes caused by water stress. Ahmad et al. (1999) used a chlorophyll meter coupled with a DGPS to map nitrogen stress in corn. A multispectral radiometer was employed to detect crop salinity stress.

However, there is a particular interest to complete and improve in terms of time and data collection amount these methodologies using faster and non-destructive techniques. Optoelectronic tends to provide less accurate measurements than conventional laboratory analysis, facilitating the collection of larger amounts of data using cheaper and less time-consuming methods and reducing any accuracy deficit.

3. Optoelectronic applications

3.1 Signal acquisition

Optoelectronic is the study and the application of electronic devices that source, detect and control light.

Optoelectronic devices impact many areas of society, from simple household appliances and multimedia systems to communications, computing, spatial scanning, optical monitoring, 3D measurements and medical instruments (Sergiyenko, 2011). It is a complex multidisciplinary system that brings together different aspects of image analysis, machine vision, electronics and computer science for the acquisition and management of images (Jain et al., 2005; Steger et al., 2007). It represents one of the most evolutionary and sophisticated methodology between the electronic applications.

The application of these innovative optical technologies in medicine, biology, agriculture, environmental sciences and public health has emerged as one of the new paradigms in today's knowledge economy. This convergence between optical and biosciences is due to the recent significant advances of photonics and biotechnologies driven by the various health, environment, and defence challenges faced by humanity at the beginning of 21st century (Tanev et al., 2008).

The photonic is the science and technology of generation, manipulation, and detection of photons, quantum units of light. Photonic is related to electronic in that it is believed that photons will play a similar central role in future information technology as electrons do today. Therefore, this discipline has become the established general term for all techniques dealing with the interaction between biological items and photons. This refers to emission, detection, absorption, reflection, modification, and creation of radiation from biomolecular, cells, tissues, organisms and biomaterials. Photonics utilizes photons instead of electrons to transmit, process, and store information and thus provides a tremendous gain in capacity and speed in information technology. Photonics is an all-encompassing light-based optical technology that is being hailed as the dominant technology for this new millennium (Prasad, 2003).

A new extension of photonics is biophotonics, which involves a fusion of photonic and biology (Birge, 2004). Biophotonic includes all the technological and engineering disciplines that use the electromagnetic radiation as the main carrier of information (light=photos, in all its extension and bands) in earth sciences and biosystems applications. It offers great hope for the early detection of diseases and for new modalities of light-guided and light-activated therapies. Also, biology is advancing photonics, since biomaterials are showing promise in the development of new photonic media for technological applications (Prasad, 2003).

3.1.1 Nature of light

Light is an electromagnetic field consisting of oscillating electric and magnetic disturbances that can propagate as a wave through a vacuum as well as through a medium.

Electromagnetic spectrum comprises of radio waves, microwaves, infrared rays, visible light, ultraviolet rays, X-rays, and gamma rays (Fig. 3.1.1.1).

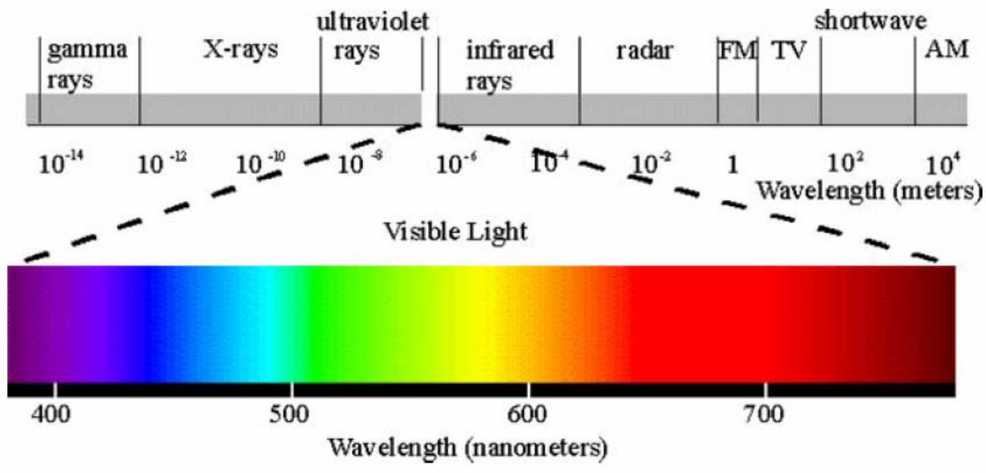

Figure 3.1.1.1: Electromagnetic spectrum (from Kaiser, 1996).

The electromagnetic spectrum is the range of all possible frequencies of electromagnetic radiation and represents the characteristic distribution of electromagnetic radiation emitted or absorbed by that particular object. It extends from low frequencies used for modern radio communication to gamma radiation at the short-wavelength (high-frequency). The types of electromagnetic radiation are broadly classified into the following classes: gamma radiation, X-ray radiation, ultraviolet radiation, visible radiation, infrared radiation, microwave radiation and radio waves. This classification goes in the increasing order of wavelength, which is characteristic of the type of radiation. While, in general, the classification scheme is accurate, in reality there is often some overlap between neighbouring types of electromagnetic energy (http://en.wikipedia.org/wiki/Electromagnetic_spectrum).

Depending on the optical regions, different units are used to characterize the wave. For the visible region, the common practice is to use the nm [or angstrom (Å)=10^{-1} nm] unit of wavelength or cm^{-1} unit of wave number. For the near-IR to mid-IR region, one often uses the wavelength in micrometers or microns (mm). From the mid-IR to far-IR region, one uses the wave number in cm^{-1} to characterize a wave (Prasad, 2003). Figure 3.1.1.2 defines the wavelength of a wave.

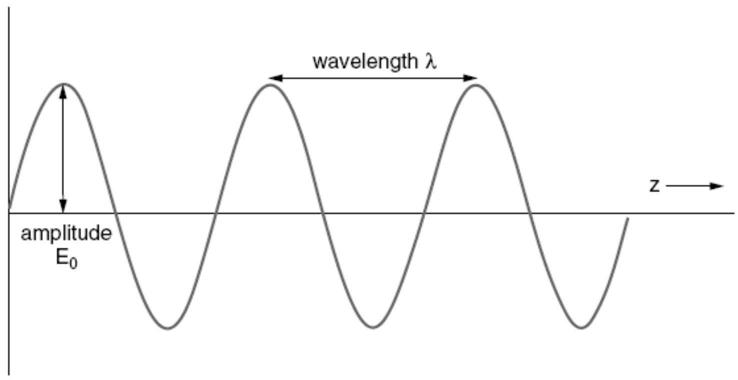

Figure 3.1.1.2: Schematic of a wave defining its wavelength reported by (from Prasad, 2003).

The interaction of light at the molecular level produces absorption, refraction, reflection, and scattering during the propagation of light through a bulk sample crating various photophysical and photochemical processes produced in the excited state generated by light absorption (Prasad, 2003).

These manifestations also play an important role in understanding the interaction of a biological bulk specimen such as a tissue with light. A major branch of interaction between light and matter is spectrophotometry, which involves the study of a transition between quantized levels.

3.1.2 Biophotonics in the agro-environment

The use of biophotonic in agricultural engineering discipline is growing in relation to the high innovation rate (based on high-tech sectors: optics, electronics, computers and algorithms) and the needs of research, production monitoring and control systems, highly informative, multiparametric, rapid and non destructive, for applications in the field (on-the-go) or in the process line (on-line) (Menesatti, 2010). Numerous studies have been analyzed and reported for agricultural applications in: robotics (vision), tractors and self-propelled machines (automatic or assisted guide), agricultural field machines (fertilizers and pesticide precise distribution), test and certification of operational characteristics and work quality, post-harvest machines (fruit selection based on external and internal qualities), quality of agricultural products (meat, fruit and vegetables, fish) and food (cheese, bread). In particular, n agriculture biophotonic research has made it possible to examine the quality of foodstuffs and the effect of different measures on them (Oikarinen, 1996).

The optoelectronic play an important role in agriculture and food industry for the rapid, non destructive and objective detection of quality represented by the organoleptic properties (Guidetti et al., 1998; Menesatti, 2000).

Among such a variety of applications this Ph.D. activities considers two of the most innovative technical-methodological sector of application: spectrophotometry and thermal analysis which have been reported in experimental applications published all on international ISI journals.

3.1.2.1 Visible (VIS) and Near Infrared (NIR) spectrophotometry

The study of radiation absorbed or reflected (i.e. determination of absorption or reflectance spectrum with spectrophotometers) is a valid and quantitative method to characterize and identify many compounds. Generally, spectrophotometry is based on the ability of the material to absorb or reflect a certain amount of incident light energy.

Spectral-based systems can be separated into two classes: 1) probe or punctual systems; 2) imaging systems. The first type provides an averaged measure of spectral values of the area acquired by the probe, integrating sphere or other devices. The second system acquires a whole sample image, extracting the spectrum for each pixel (Urbani et al., 2002).

The spectrophotometer is an instrument for spectral components acquisition of reflected or transmitted light by objects. The measured value is mediated on object area framed by the capture probe. Within this area, the system is unable to distinguish the spectral contributions of different structures. The system especially consists of a spectrometer (passive optical device) and the electronic equipment to generate light and acquisition, elaboration and transmission cable of the light signal converted into an electrical signal. A timely example of a system is illustrated in Figure 3.1.2.1.1.

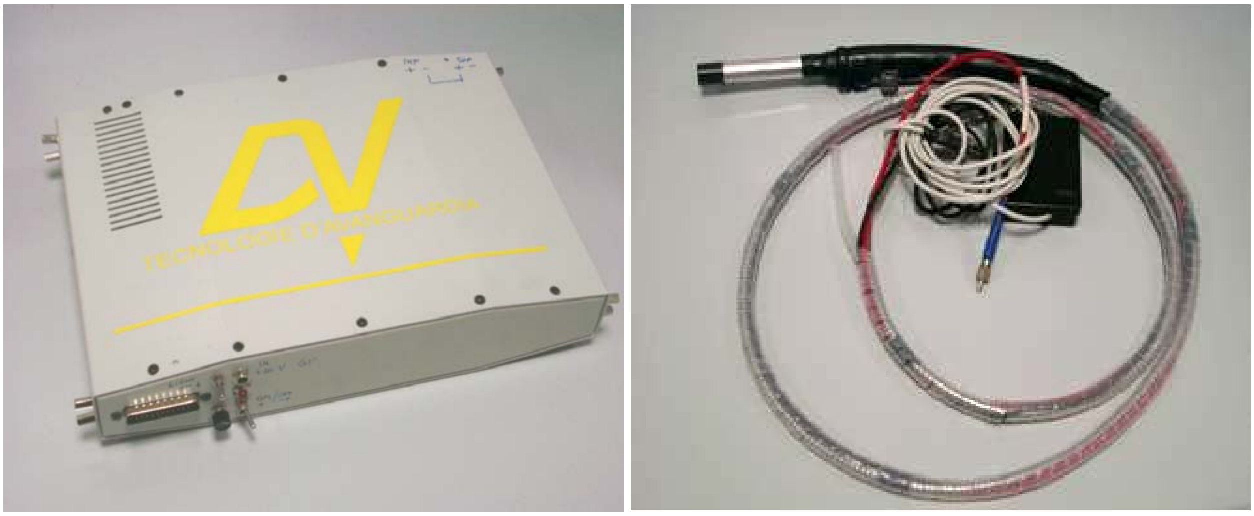

Figure 3.1.2.1.1: Example of a portable spectrophotometer.

The system has a spectral range between 280 nm and 1180 nm. Thus, this spectrometer is sensitive to ultraviolet (UV: 280-400 nm), visible (VIS: 400-700 nm) and near infrared (NIR:

700-1180). For the full range of sensitivity, the system provides spectral data on average every 3 nm (300 wavelengths), with a maximum dynamic of signal of 14 bits (65,000 levels). The reflectance is also influenced by the spectrum of the illumination light. Generally, illumination systems are based on halogen lamps with dichroic (non-reflective infrared) or aluminium base (reflecting infrared spectrum) to illuminate the target with a full spectra white light.

The imaging systems with VIS-NIR and NIR spectrophotometers (Fig 3.1.2.1.2) were composed of four parts: (1) a sample transportation plate (spectral scanner DV, Padova, Italy) (common to both systems); (2) a collimated illumination device (Fiber-lite, Dolan-Jenner, MA, USA) with a 150 W halogen lamp and an illumination opening in the optical fibre measuring 200 mm long by 2 mm width, positioned at 45° to the transportation plate for minimum light divergence (common to both systems); (3) an imaging spectrographs (VIS-NIR: ImSpec V10; NIR: ImSpec N17, Specim Ltd, Oulu, Finland) coupled with a standard C-mount zoom lens; and (4) digital camera (VIS-NIR: Teli charge-coupled device (CCD) monochrome camera, Toshiba-Teli CS8310BC; NIR: Pixelvision SU128 InGaAs IR camera).

Figure 3.1.2.1.2: Visible-Near Infrared (VIS-NIR) spectrophotometer for the spectral images acquisition.

The imaging spectrometers were used to acquire images ranging from 400 to 970 nm and from 1000 to 1700 nm respectively. The two spectrographs are based on a patented prism-grating-prism (PGP) construction (a holographic transmission grating). The incoming line image (frame) was projected and dispersed onto the 2D CCD. Each frame contained the line pixels in one dimension (spatial axis) and the spectral pixels in the other dimension (spectral axis), providing full spectral information for each line pixel. The reconstruction of the entire hyperspectral image of the sample was performed by scanning the sample line-by-line as the transportation plate moved it through the field of view. The resolution of the line image was 700 pixels by 10 bits for the VIS-NIR and 128 pixels by 12 bits for NIR. The system was operated in a dark laboratory to minimize interference from ambient light. All spectral values were expressed in terms of relative reflectance (R), following equation 1:

$$R = \frac{r_s - r_b}{r_w - r_b}$$

where R is the relative reflectance of the sample at a given wavelength; rs is the absolute signal value (radiance) measured for the sample at the wavelength; rb is absolute signal value (radiance) measured at each wavelength for the black background (noise); rw is absolute signal value (radiance) measured at the wavelength for a standard white background (100% of reflectance).

3.1.2.2 Thermal analysis

Temperature measurement is an important aspect in any industrial process and infrared and thermal applications have revolutionized the concept of temperature measurement. Temperature measurements were generally performed using thermometers, thermocouples, thermistors and resistance temperature detectors. These instruments can only determine temperature at specific points and most of these instruments need to establish a contact with the material, while, infrared thermal imaging is a non-contact and non-destructive technique which provides temperature mapping of a material. For these reasons the use of infrared thermal imaging is widely increasing in many fields (Vadivambal & Jayas, 2010). All objects above 0 K (−273.15°C) emit infrared rays which are part of the electromagnetic spectrum. The wavelength of infrared rays is in the range of 0.78– 1,000 µm. The infrared region is further divided into different regions: near infrared (0.75–3 µm), mid infrared (3–6 µm), far infrared (6–15 µm), and extreme infrared (15–1,000 µm) (Meola & Carlomagno, 2004). The intensity of radiation emitted by an object is a function of its surface temperature, i.e., the higher the temperature of the body, the greater is the intensity of infrared radiation emitted by the object. Thermal imaging is a technique which converts this radiation emitted by an object into temperature data without establishing contact with the object (Vadivambal & Jayas, 2010).

Infrared thermography (IT) is a non-destructive evaluation method image-based measuring specific electromagnetic radiation emitted by any object according to the Stefan-Boltzman's and Planck's laws (Maldague, 1994). All objects emit heat (energy) waves. If an object is cold, its molecules vibrate slower and energy of longer wavelengths is emitted. When the temperature of the object rises, its molecules vibrate faster and the wavelength becomes shorter (Antonucci et al., 2011).

Among the different image analysis techniques and technologies, thermography has the capability to associate to the image information, the thermal punctual information, which is the temperature of each single pixel, in order to operate comparison between objects inside the same image. Thermal imaging devices provide the observer with instruments that can collect (just like a video or still camera) and convert the thermal infrared radiation emitted (and reflected) by objects into images that can be seen on a view screen or computer display (Menesatti et al., 2007).

Thermal imaging has a wide application in various fields such as civil engineering, industrial maintenance, aerospace, medicine, pharmacy, and veterinary.

The application of thermal imaging is gaining popularity in agriculture and food industry in recent years. The major advantages of thermal imaging are non-contact, non-invasive, and rapid technique which could be used for online applications. The thermal cameras are easy to handle and highly accurate temperature measurements are possible. With the thermal imaging, it is possible to obtain temperature mapping of any particular region of interest with fast response times which is not possible with thermocouples or other temperature sensors which can only measure spot data. Repeatability of temperature measurements is high in thermal imaging. Also, thermal imaging does not require an illumination source unlike other imaging systems (Vadivambal & Jayas, 2010).

Infrared thermal imaging system comprises of thermal camera equipped with infrared detectors, a signal processing unit and an image acquisition system, usually a computer.

All objects with a temperature greater than absolute zero (–273°C) emit infrared radiation. The emissivity, absorptivity, transmissivity, and reflectivity of infrared radiation vary for different materials. In general, the objects which are good absorbers of infrared radiation are also good emitters (Manickavasagan et al., 2005). The infrared detectors absorb the infrared energy emitted by the object and convert it into an electrical impulse. The electrical impulse is sent to the signal processing unit which translates the information into thermal image. Most of the thermal imaging devices scan at a rate of 30 times per second and can sense temperature ranging from −20 to 1,500°C, but the temperature range can still be increased by using filters (Meola & Carlomagno 2004). Detectors are the most important part of thermal imaging system which converts the radiant energy into electrical signals proportional to the amount of radiation falling on them. Thermal imaging devices can be classified into uncooled and cooled. The uncooled thermal imaging device is the most common one and the infrared detector elements are contained in a unit that operates at room temperature. They are less expensive but their resolution and image quality tend to be lower than the cooled device. In the cooled thermal imaging device, the sensor elements are contained in a unit which is maintained below 0°C. They have a very high resolution and can detect temperature difference as low as 0.1°C but they are expensive. Cooled thermal imaging devices are used in military and aerospace applications. An infrared imaging system is evaluated based on thermal sensitivity, scan speed, image resolution, and intensity resolution (Vadivambal & Jayas, 2010). Thermal imaging has a potential application in many operations involved in agriculture, starting from assessing the seedling viability, estimating soil water status, estimating crop water stress, scheduling irrigation, determining disease and pathogen affected plants, estimating fruit yield, and evaluating maturity of fruit and vegetables. This section elaborates on studies conducted to determine potential use of thermal imaging in agriculture (Vadivambal & Jayas, 2010). This method is suitable for making quality determination of surface temperature than quantitative measurement (Davis & Lettington 1988). This technology can be used in all agricultural materials and processes, where heat is generated or lost in space and time (Hellebrand et al. 2002). Small variations (below 1°C) can also be successfully measured with proper equipment and methodology.

3.2 Signal analysis

3.2.1 Multivariate statistical analysis

Multivariate analyses are generally divided into two main categories: unsupervised and supervised. For unsupervised techniques, grouping or clustering methods for multivariate elements (x-block) are based on functional relationships among the same elements (distances, variances). They do not need for an a priori knowledge of the class categories. Differently, in supervised techniques, the class attribution is given by a single or multiple variables (y-block). In this way, multivariate methods are forced to cluster into a priori established classes. Unsupervised methods are mainly applied in an exploratory sense, when the aim is to analyze or visualize non-forced aggregating relationships (unsupervised) among elements (Forina, 2006).

Concerning supervised techniques, it is possible to distinguish two main analytical approaches: modelling and classification. Supervised methods are derived from the observation and then the use of known classes, called the training set. The derived classification criteria can then be used to classify each new object within a test set. This can be applied for both classification and the computing of efficiency parameters. Classification analysis needs a decision rule, called the "classification criterion", to distinguish objects into classes on the basis of selected quantitative features (Jayas et al., 2000). For modelling, it is instead possible to attribute objects not only into one or more classes but also to none (i.e. in this case, the object is an outlier). Modelling techniques calculate the "prediction probability" with a classification threshold for each modelled class. The modelling efficiency is indicated by statistical parameters such as "sensitivity" and "specificity".

Sensitivity represents the percentage of the objects of a category accepted by the modelled class. Specificity is the percentage of objects different from the modelled classes, as rejected by this classification criterion. On the other hand, for the classification, a matrix of correct classification can be used (Forina, 2006). The statistics used to investigate ratios and shape indices are normally descriptive and represented by simple regression (Li et al., 2004), ANOVA (Brewer et al., 2007), PCA and canonical discriminant analysis (Brewer et al., 2007). Many other studies uses instead shape-based methods in association with PCA (Ohsawa et al., 1998; Paulus & Schrevens, 1999; Currie et al., 2000; Cannon & Manos, 2001; Beyer et al., 2002; Goto et al., 2005; Morimoto et al., 2005; Brewer et al. 2006). This is because different shapes exhibit a certain level of quantitative variation related to genotypic and environmental effects. While shape can be categorized in some way for species, sub-species, cultivar, merceologic classes, crops, etc., the quantitative variation in fruit shape can be analysed by methods based on classification and modelling such as PLS-based (PLS, PLSDA; Sjöström et al., 1986; Sabatier et al., 2003; Bylesjo et al., 2006; Tominaga, 2006; Menesatti et al., 2008; Antonucci et al., 2011;

Costa et al., 2011), soft independent modelling of class analogy (SIMCA; Bylesjo et al., 2006; Tominaga, 2006; Casale et al., 2007; Aguzzi et al., 2009a, b), clustering of the Fourier coefficients (Costa et al., 2009a, b) and standard non-linear Bayesian discriminant analysis (Blasco et al., 2009). Also, ANNs have been widely used for quantifying the variation in the shape of fruit (Morimoto et al., 2000). ANNs are very effective in many applications and are particularly useful as generalized non-linear regression tools (Costa et al., 2009b). They can perform arbitrary non-linear mappings in patterns of information.

4. In-field soil preparation

The basic objectives of in-field management of agricultural inputs are to increase profitability of production, improve product quality and protect the environment. As reported by Adamchuk et al. (2004) information about the variability of different soil attributes within a field is essential for the decision-making process. The inability to obtain soil characteristics rapidly and inexpensively is one of the biggest limitations of precision agriculture. It is observed that generally, data are collected, processed and stored in different physical location making the analysis operations very slow and not suitable to managing agricultural practices to be carried out immediately (e.g., crop plantation, cropping decision, irrigation scheduling, fertilization rates, etc.).

One of the most critical aspects of soil testing is actually obtaining representative soil samples (i.e. collected with adequate spatial density at the proper depth and during the appropriate time) (Adamchuk et al., 2004). In addition, with mechanization and intensification of agriculture in Europe, fields and farms are becoming larger. Nowadays, random, adaptive and grid sampling techniques are often used. In random sampling, soil cores are obtained from random locations within the field. In adaptive sampling, selected locations depend on prior information. Grid sampling, on the other hand, involves systematically collecting samples from predetermined points in the field.

Numerous researchers and manufacturers have attempted to develop on-the-go soil sensors to measure mechanical, physical and chemical soil properties (Sonka et al., 1997). However, currently inputs, such as for example fertilizer, pesticides and irrigation, are applied uniformly despite numerous variations in the soil type and crop density (West et al., 2003). Recent developments in agricultural machinery have made it possible to spatially adjust farm applications (Secher, 1997). As reported by Hellebrand et al. (2002) since precision farming utilises higher spatial resolution than traditional farming, extensive soil and plant data have to be managed. For these reasons fast measuring techniques (i.e., sensor-based managing; see paragraph 2.3.2) are looked for, which provide information on the state of soil and plants. The main advantage of these methods is that they are usually non-destructive. Moreover, knowledge on heterogeneity of the fields, in particular on soil spatial variability, is essential for a good agricultural practices management. Information on the state of the soil and plants may be getting as on-the-go monitoring of variable quantities in line with immediate responds of the machine system. The other possibility is the separation in time of sensing and control action. The number of data sets increases in dependence with the spatial resolution: the smaller the sites, which are treated uniformly within the field, the more data sets must be supplied.

Generally, in-field sensor-based managing is needed in site-specific crop production for the control of soil and plant properties, which are spatially variable. Essential for economy and environment, and which cannot be recognised by the farmer during field-work immediately (Hellebrand et al., 2002).

Appropriate sensors mounted on a ground-based vehicle (Fig. 4.1) could have a high resolution, allowing the possibility to collect data for soil survey (McKenzie & Austin, 1993), soil evaluation for sustainable use (Fu et al., 2000) and specific agricultural planning and management (Lark, 1999).

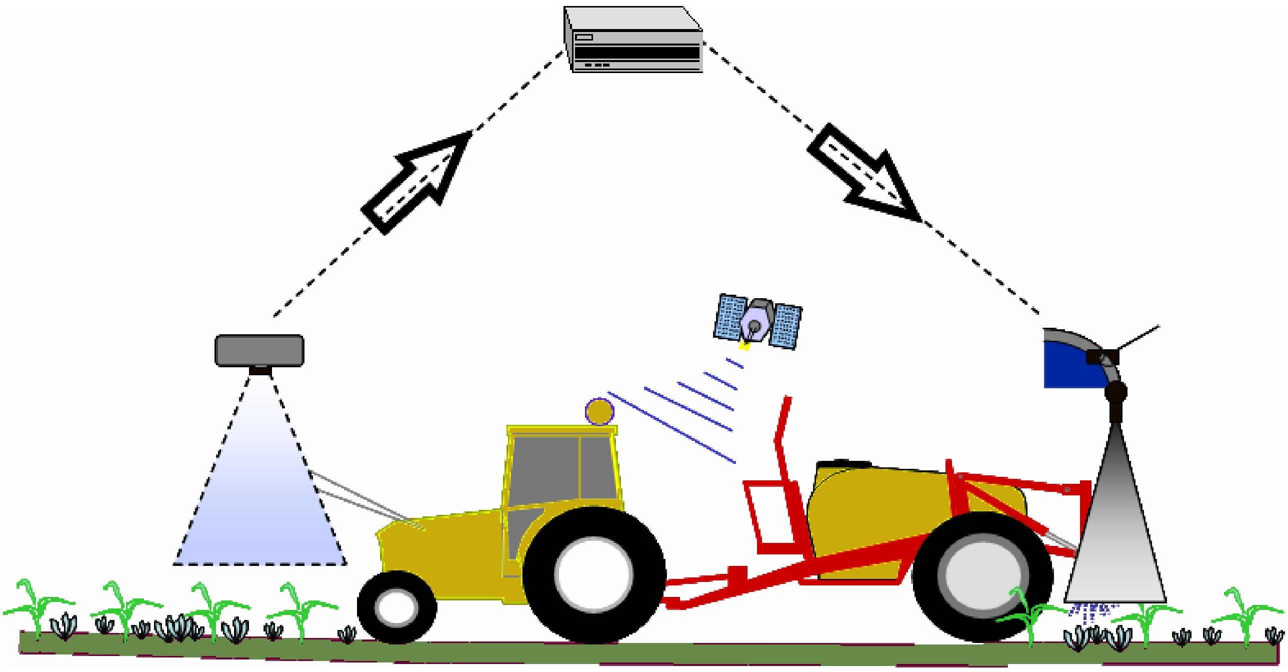

Figure 4.1: Example of sensors mounted on a ground-based vehicle for in-field applications (adapted from Oberti, 2009).

In this context, an important agro-environmental production parameter to rapidly monitor directly in field is the soil water content, which, if not monitored regularly and intensively, could represent a serious problem in farm management practices. The effects of crop management practices on soil water content may vary geographically and temporally depending upon environmental and climatic conditions (Doran & Smith, 1987). Greater knowledge of the short- and long-term effects of various agricultural management practices on soil biological, chemical, and physical properties is needed to assess the contribution of these practices to sustainable land management (Franzluebbers et al., 1995).

4.1 Soil water content estimation

As all soil properties, water content is variable in three dimensional space and in time. This variability complicates the tasks of measuring, modelling, estimating or forecasting of soil properties. Variability has been dealt with in numerous ways, including the collection of a great number of samples and through various statistical approaches (Hignett & Evett, 2008) but this is too expensive and is not possible in some circumstances.

The standard method of soil water content measurement involves taking a physical sample of the soil, weighing it before any water is lost, and drying it in an oven before weighing it again. The mass of water lost on drying is a direct measure of the soil water content. This measure is normalized either by dividing by the oven-dry mass of the soil sample, or by converting the mass of water to a volume (by dividing the mass of water by the density of water) and dividing this volume of water by the volume of the sample.

The mass basis water content of a field soil can be used for comparative purposes and is useful when soil volume changes, as with tillage. However, for most irrigation, cropping decisions, crop water use, and water use efficiency work, what is required is the volume of water in a certain volume of soil or the equivalent depth of water in a certain depth of soil. Both of these require knowledge of the volumetric water content. The standard measures of soil water only apply to the position in the soil that was sampled. A single such sample is of limited value to an irrigator, crop or environmental scientific or hydrologic managements. Moreover, the use of direct soil sampling is destructive of the field, labour intensive, is often slow, not timely and may be costly. Also, by its nature, direct sampling cannot measure the water content in the same place twice. For work that depends on the change in water content with time, this fact adds further variability to the data due to the inherent small scale variability of water content (Hignett & Evett, 2008).

In the past few decades, a great number of automated techniques for point measurement of soil water content have been developed and tested because of the important role soil water content plays in guiding the management of for example irrigation and drainage (Luhr & Kleisinger, 1998; Evett, 2008). Generally, as reported by Chow et al. (2009) those techniques can be classified into the various categories: i) tensiometers (a plastic tube with a porous ceramic cup attached at one end and a vacuum gauge or a pressure transducer on the other end, measuring the soil water tension or suction in units of kPa; ii) resistance blocks (two electrodes imbedded in a porous material determining soil water according to electrical resistance measured with an alternating current bridge); iii) heat dissipation type (derives soil water by measuring how much heat is dissipated in a ceramic medium buried in a soil); iv) dielectric sensors (obtain soil water by measuring the apparent dielectric constant of a soil); v) connector type sensors (based on dielectric properties of a soil); vi) neutron method (consisting of a radioactive fast neutron source probe and a helium-3 detector, estimating soil water by the recorded count of thermal neutrons which are thermalized by the hydrogen present in the soil).

All these methods are destructive and time-consuming. A new method to overcome these difficulties and indirectly determine soil water content by measuring its temperature changes after heating is represented by the active infrared thermal analysis.

4.1.1 Why monitor soil water content?

Soil water content is an important factor used in agriculture to make decisions regarding land managers making decisions concerning livestock grazing patterns, crop planting, irrigation scheduling, and soil stability for agricultural machinery operations (Qiu et al., 2001). Moreover, the spatial variation of soil water content is a necessary and preliminary part for parametric soil and land survey (McKenzie & Austin, 1993), spatial prediction of soil moisture (Ladson & Moore, 1992; Grayson & Western, 1998; Lark, 1999; McKenzie & Ryan, 1999), soil and land evaluation for sustainable use (Fu et al., 2000), specific farm planning and management (Odeh et al., 1994; Lark, 1999), hydrologic modelling and watershed management (Western & Grayson, 1998) and irrigation scheduling (Jones, 2004).

Cultivation of crops involves several activities undertaken by farmers over a period of time. These activities or tasks are referred to as agricultural practices (e.g., preparation of soil, sowing, adding manure and fertilisers, irrigation, protecting from weeds, harvesting and storage). The preparation of soil is the first step before growing a crop. One of the most important tasks in agriculture is to turn the soil and loosen it. The process of loosening and turning of the soil is called tilling or ploughing. If the soil is very dry, it may need watering before ploughing. For this reason it is very important to know the soil water content as first step to prepare soil for crop planting.

Also in the irrigation scheduling it is fundamental to fast detect the soil water content. In fact, irrigation is an increasingly important practice in the management of valuable water resources in agricultural regions. The increasing worldwide shortages of water and costs of irrigation are leading to an emphasis on developing methods of irrigation that minimize water use (maximize the water use efficiency). The advent of precision irrigation methods such as trickle irrigation has played a major role in reducing the water required in agricultural and horticultural crops, but has highlighted the need for new methods of accurate irrigation scheduling and control (Jones, 2004).

In addition land use, an alternative attribute that is easily obtained, also plays an important role in controlling spatial patterns of soil moisture by influencing the infiltration, runoff and evapotransipiration, particularly during the growth season (Fu & Chen, 2000; Fu et al., 2000).

4.2 Case study

For the in-field soil preparation phase a rapid soil water content detection by active infrared thermal methods was developed. This case study was published in an international peer-reviewed journal with impact factor.

The aim was to investigate the suitability of active infrared thermography and thermometry, combined with multivariate statistical partial least squares analysis, as rapid soil water content detection techniques both in laboratory and in-field. These techniques allowed fast soil water content measurements helpful in both agricultural and environmental fields (Fig. 1.1).

4.2.1 Antonucci F, Pallottino F, Costa C, Rimatori V, Giorgi S, Papetti P & Menesatti P (2011) Development of a rapid soil water content detection for in-field applications by active infrared thermal methods. Sensors, 11, 10114-10128. (IF2010=1,771)

Abstract

The aim of this study was to investigate the suitability of active infrared thermography and thermometry in combination with multivariate statistical partial least squares analysis as rapid soil water content detection techniques both in the laboratory and the field. Such techniques allow fast soil water content measurements helpful in both agricultural and environmental fields. These techniques, based on the theory of heat dissipation, were tested by directly measuring temperature dynamic variation of samples after heating. For the assessment of temperature dynamic variations data were collected during three intervals (3, 6 and 10 s). To account for the presence of specific heats differences between water and soil, the analyses were regulated using slopes to linearly describe their trends. For all analyses, the best model was achieved for a 10 s slope. Three different approaches were considered, two in the laboratory and one in the field. The first laboratory-based one was centred on active infrared thermography, considered measurement of temperature variation as independent variable and reported r=0.74. The second laboratory–based one was focused on active infrared thermometry, added irradiation as independent variable and reported r=0.76. The in-field experiment was performed by active infrared thermometry, heating bare soil by solar irradiance after exposure due to primary tillage. Some meteorological parameters were inserted as independent variables in the prediction model, which presented r=0.61. In order to obtain more general and wide estimations in-field a Partial Least Squares Discriminant Analysis on three classes of percentage of soil water content was performed obtaining a high correct classification in the test (88.89%). The prediction error values were lower in the field with respect to laboratory analyses. Both techniques could be used in conjunction with a Geographic Information System for obtaining detailed information on soil heterogeneity.

Keywords: soil moisture; Partial Least Squares; thermography; thermometry; sensor techniques; irradiance; heat dissipation

4.2.1.1 Introduction

Recently, the need to measure in-field the variability of soil characteristics has increased following both sensor engineering developments, as well as the necessity to apply innovative crop management systems (Castrignanò et al., 2002). Changes in soil characteristics such as cation exchange capacity, organic carbon and water content may occur as the sampling point changes, even by few cm. A fine analysis carried out with conventional methods would require a lot of manual and laboratory work and incur high costs for the numerous samplings needed (Hummel et al., 2001). Researchers have investigated several approaches in order to automate these procedures (Sudduth & Hummel, 1996) and to overcome the critical aspect of soil management in collecting representative samples (Wollenhaupt et al., 1997). For these reasons, methods increasing the acquisition of a high number of sample variables at a relatively low cost and time, such as vehicle-mounted optical sensing devices, represent promising application perspectives (Committee on Assessing Crop Yield, 1997). These multi-devices systems could include mobile instruments (i.e., visible-near and near infrared spectrophotometers, infrared thermometers and thermocameras). These could be used to measure different surface-layers soil parameters such as reflectance, absorbance and temperature.

An important soil property is the spatial variation of water content measured at a proper depth and time (Huisman et al., 2002). The description of spatiotemporal soil water content (SWC) changes requires understanding of both spatial and time variability but results are relevant for many applicative agricultural contexts such as for example: trafficability, soil compactness and crop hydric stress (Castrignanò et al., 2003). Generally, the most common techniques to analyse SWC use punctual, destructive, expensive or time-consuming procedures (Edmeades et al., 1985; Roth et al., 1992), mainly based on opto-electronic, gravimetric, nuclear, electromagnetic, tensiometric and hygrometric processes (Zazueta & Xin, 2004). Within the opto-electronic methods, near infrared (NIR) spectrophotometry is one of the most used to calculate SWC in surface and subsurface layer, but its results show a tendency to underestimate values at higher water levels (Sudduth & Hummel, 1993; Sudduth et al., 2001). Another similar approach was carried out by Maltese et al. (2010). In this work the technological development of imaging sensors acquired in the visible (VIS), NIR and thermal infrared (TIR), renewed the research interest in setting up remote sensing based techniques aimed at retrieving SWC variability in the soil-plant-atmosphere system (SPA). The soil thermal inertia method (soil resistance to surrounding temperature change) is an additional method widely used to estimate soil moisture from TIR and VIS bands for bare soil (Lu et al., 2009; Minacapilli et al., 2009). This technique requires readily available soil characteristics such as soil texture and bulk density. Among the gravimetric methods, the oven-drying technique is probably the most widely used. This method is considered as the standard for the calibration of all other soil moisture determination techniques. Nevertheless, it has some disadvantages, being a destructive test requiring sample removal and making it impossible to measure the water content at exactly the same point at a later date (Mckim et al., 1980). Another method is neutron scattering. This method obtains a profile of moisture distribution but it has some disadvantages such as radiation hazards, insensitivity near soil surface, insensitivity to small variations in moisture content at different points, and variation in readings due to soil density variations, which may cause an error rate of up to 15% (Verstraeten et al., 2008). Among the electromagnetic techniques there are those that measure the soil electrical resistivity, obtaining hence its water content. In this case, the disadvantages regard the instable calibration over the time affected by ionic concentration and the cost of equipment (Zazueta & Xin, 2004).

Another widely used method for small spatial scale estimates of SWC is the measurement of soil thermal properties such as the heat dissipation technique and the heat pulse technique (Young et al., 2008). This method, contrary to the other previously reported ones, is non-destructive, and requires a small sample size which provides good spatial resolution, it is suitable for laboratory and field applications, does not need any calibration and conversely to the known electromagnetic techniques, it does not modify the soil's electric properties. These are over a certain period of time permanently modified invalidating future readings (Zazueta & Xin, 2004). These indirect methods exploit changes in soil thermal properties due to variation of SWC. In soil, the driving force which regulates its temperature is the water content, being its specific heat (i.e., 1 J/g °C) higher than that of the other substances that make up the soil itself (0.19–0.35 J/g °C). In fact, the same amount of heat supplied to certain soil samples with different water contents can lead to different temperature differentials. Commercial heat dissipation sensors are broadly available. They basically consist in a heat source (usually a heated needle) and temperature sensors, immersed in a porous ceramic that equilibrates with the surrounding soil at a given water content. The needle is heated and the rate of heat dissipation is measured by the temperature sensors (Bittelli, 2011). However, sensor use is limited by the need of calibration for any type of soil and by the long time to reach hydraulic equilibrium with the surrounding soil. The time required to reach the hydraulic equilibrium between heat dissipation sensors and soil depends on both the magnitude of the SWC and the hydraulic conductivity. Typically this equilibration time is on the order of minutes or tens of minutes (Campbell Scientific Inc, 2009).

In order to overcome the limits of heat dissipation sensors, in this study we propose the use of a new technique based on the same underlying theory of the heat dissipation methods. Unlike heat dissipation sensors, we propose to directly measure temperature changes of soil samples, after heating, by using active infrared thermography and thermometry. The assumption is that these techniques could lead to the development of a faster SWC measurement system and could represent informative and non-destructive tools to remotely assess the dynamic variation of soil temperature (Newton et al., 1983; Maldague, 2002). Moreover, these could be implemented on vehicle-mounted systems to shorten sampling time and the amount of soil surveyed. The main principle of these applications concerns the measurement of the thermal infrared spectrum of electromagnetic radiation emitted by soil samples depending on their temperature (Maldague, 1994; Rahkonen & Jokela, 2003). For in-field applications this technique should measure surface soil (0–60 cm) temperature, that is influenced by soil-atmosphere interactions. This aspect makes unsuitable the use of calibration curves to relate temperature to SWC as physical or empirical relationships, which describe all the soil-atmosphere interactions. In fact the general model describing the soil-atmosphere interaction is given by the energy balance equation (Campbell & Norman, 1998):

$$R_n + M - H - \lambda E = G \tag{1}$$

where R_n is the net radiation at soil surface, M represents the supply of energy to the surface by metabolism or absorption of energy by photosynthesis, H is the sensible heat flux, λE is the latent heat flux by evapotranspiration and G is the soil heat flux.

Adapting the energy balance Equation (1) to the proposed study and analyzing the water content on a bare soil after primary tillage and exposed to soil irradiance, the M becomes negligible and G is equal to:

$$G = G_s + G_l \tag{2}$$

where G_s is the heat variation of soil surface and G_l the heat flux in the soil by contact. The surface thermal variation will be related to G_s, H, λE and G_l. In this case, these parameters will be dependent on agro-pedological and meteorological parameters such as air temperatures and humidity, SWC, irradiance, wind regimes, soil water potential and soil roughness. The deterministic modelling of the environmental variables influencing the physical process which is developing in such a short time of analysis (few seconds) would have been very complex.

For the above mentioned reasons the system could be approached in a statistical way and the estimation of SWC innovatively implemented by using a multivariate analysis (Antonucci et al., 2011; Menesatti et al., 2010), taking into consideration different soil thermal properties and meteorological parameters as input variables. Differently from deterministic models, stochastic ones do not explain the underlying physical processes generating the observations and the model randomness. Modelling spatiotemporal distributions, resulting from dynamic processes and evolving in both space and time, is critical in hydrology and soil science. Statistical spatiotemporal models provide a probabilistic framework for data analysis based on joint spatial and temporal dependence among observations (Castrignanò et al., 2003).

In this study, a multivariate statistic approach (Partial Least Squares regression, PLS, and Discriminant Analysis, PLSDA) is used to estimate the SWC with active infrared thermal methods by warming up and measuring, at different time steps, several non-factorial soil samples with different water contents. Three different hypotheses were considered, two in the laboratory and one in the field. The laboratory experiments were carried out to determine the best performing one. The latter was then chosen in order to be applied in-field. The first one tested in the laboratory is based on active infrared thermography, which considers only the measurement of temperature variation as independent (observed) variable. The second one examined in the laboratory added the irradiation of soil samples as independent variable and it was based on active infrared thermometry. Finally the in-field experiment was based on active infrared thermometry and also considered some meteorological parameters as independent variables (i.e., air temperature and relative humidity, wind speed and irradiance at soil surface).

4.2.1.2 Experimental Section

4.2.1.2.1 Laboratory Analysis

In order to develop models for the statistical interpretation of the phenomenon, according to the previously indicated thermo-physical context, a series of progressive laboratory tests were performed. These laboratory tests were developed to highlight the limits and possibilities of the techniques and chose among them the most suitable one for an in-field application.

The experimental laboratory protocol consists in warming up soil samples with different initial temperatures and water contents and in measuring for a few seconds the dynamic temperature variations. This investigation was carried out in two different steps: the first with an infrared thermocamera considering as dependent variable the percentage of water content and as independent ones the initial soil temperature and the exposition time at constant irradiance.

In a second step, an infrared thermometer was used to simplify the measuring system by introducing among the independent variables also the irradiance produced by photographic bulbs (200 W and 2,800 K) to approach in-field applications. In both cases, air temperature and air relative humidity were considered as constant.

4.2.1.2.1.1 Thermographic Analysis

Soil samples, number of soil samples (N=250), were collected from the CRA-ING experimental field (Lat. 42°06’11.00’’N, Long. 12°37’40.81’’E) at a depth of 30 cm. The operative soil status being normally unknown, different initial levels of water content and temperature were achieved by hydrating, dehydrating, warming up (stove, 40–60 °C; controlled environment, 20–25 °C) and cooling down (fridge, 2–4 °C) different plastic trays (20 × 30 cm) previously filled with soil samples.

The dynamic variation of sampled soil temperature was identified by an operator analyzing a specific thermal image area called the Region Of Interest (ROI). The temperature values were collected at four different intervals: 0, 3, 6 and 10 s. The water content (%) was expressed gravimetrically as percentage of grams (g) of water on g of dry soil (θ g, water g/dry soil g). The water content reference measurements of samples were obtained through the official oven-drying gravimetric technique (Mckim et al., 1980), by placing the sample in an oven at 105 °C until stabilization of weight.

The soil surface temperature dynamic variation was acquired using a FLIR (S40) thermocamera [Figure 4.2.1.2.1.1.1(A,B)] with the following characteristics: detector type, Focal Plane Array (FPA) uncooled microbolometer; Field Of View (FOV), 24° at distance of 1 m the FOV is equal to 0.42 × 0.31 m; Instantaneous Field Of View (IFOV), 1.3 mrad (the theoretical FOV of one pixel); image frequency, 60 Hz; spectral range, 7.5 to 13 μm; focus, automatic or manual; thermal sensitivity 50/60 Hz, 0.08 °C at 30 °C; temperature range −40–+120 °C. The emissivity (ε), the capability of an object to adsorb or emit the thermal radiation, for the soil was set equal to 0.96 (Campbell & Norman, 1998).

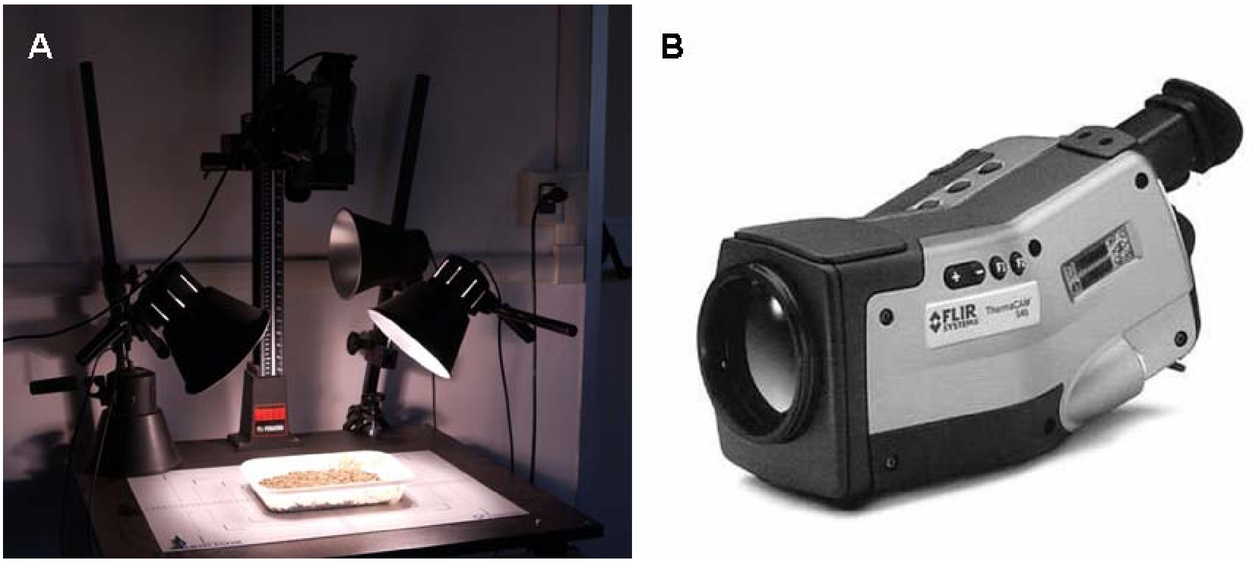

Figure 4.2.1.2.1.1.1: (A) Thermographic laboratory analysis system. Special photographic bulbs heating the soil samples in apposite plastic trays (20 × 30 cm) for the active infrared thermographic analysis. (B) Thermocamera FLIR (S40).

4.2.1.2.1.2 Thermometric Analysis

As in the previous case, soil samples (N=50) were collected in the CRA-ING experimental field at a depth of 30 cm. In addition to different initial levels of water content and temperature, different irradiance values were considered for all the samples. Irradiance was measured by a radiation sensor whose sensible element is a photodiode that converts incident radiation into a voltage (LP-9021 RAD, Delta Ohm, Padova, Italy). The signal is then acquired by a portable microprocessor-controlled multifunction quantum-photo-radiometric indicator with LCD indication (HD-9021, Delta Ohm). The sensor measures the flux of incident radiation in the spectral region spanning from 450 nm to 950 nm, ranging from 0 to 2,000 W/m^2 and having a precision of ±3.5%. The portable indicator has a resolution of 0.1 W/m^2 for values minor than 200 W/m^2 and 1 W/m^2 for values greater than 200 W/m^2.

Soil surface temperature dynamic variation was measured at four intervals (0, 3, 6 and 10 s) by an infrared thermometer measuring the amount of radiant energy emitted by the samples (IRtec P500, Eurotron, Milano, Italy). The instrument has a measurement range from −30 °C to 930 °C, a resolution of 0.1 °C and an accuracy of ±1% + 1 °C. Also in this case the water content reference measurements of samples were obtained through the oven-drying gravimetric technique (Sudduth & Hummel, 1993).

4.2.1.2.2 In-Field Analysis

Soil samples (N=40) were collected in the CRA-ING experimental field facilities during three different days and times in order to obtain a high SWC and solar irradiance variability. The samples were collected on the surface of bare soil after deep ploughing (60 cm) [Figure 4.2.1.2.2.1(A)] and primary milling (15 cm) [Figure 4.2.1.2.2.1(B)]. The measurements were carried out after ploughing just for practical reasons but the models are meant to work in pair with any soil baring system. The temperature dynamic variations were measured at four intervals (0, 3, 6 and 10 s) by the infrared thermometer [Figure 4.2.1.2.2.1(C)] on 40 different surface points of the bare soil. The temperature variation was achieved by solar irradiance and measured with the radiation sensor. At the same time air temperature and relative humidity and wind speed were collected with a weather station (Vantage Pro2™, Davis Instruments Corp., Hayward, CA, USA). The water content reference measurements were obtained through the oven-drying gravimetric technique (Mckim et al., 1980), catching the first 5 cm of the bare analyzed soils and bringing them in laboratory after temperature acquisition.

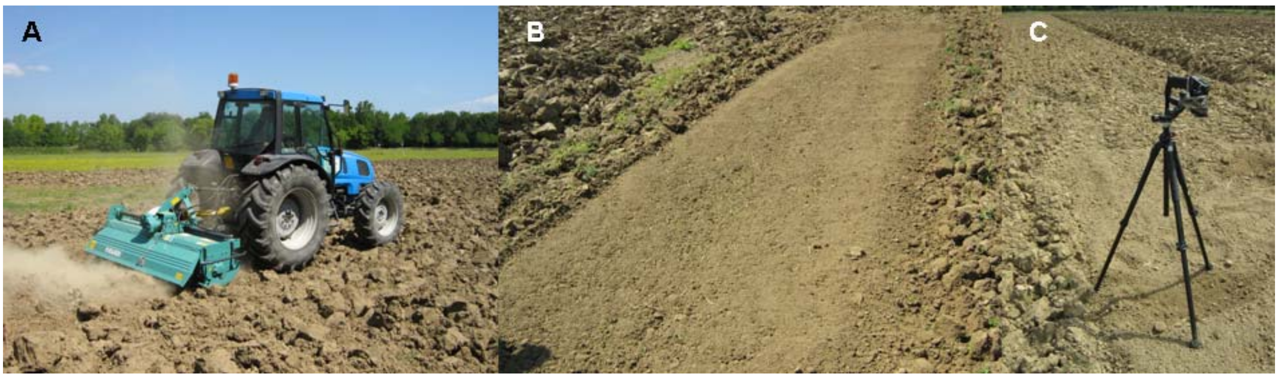

Figure 4.2.1.2.2.1: (A) Soil deep ploughing (60 cm); (B) Soil after primary tillage (15 cm).

4.2.1.2.3 Datasets Creation and Statistical Analysis

For the datasets creation, the temperature dynamic variations were collected at four intervals (0, 3, 6 and 10 s), named hereafter t_0, t_3, t_6 and t_{10}, respectively. For the presence of different specific *h*eats between water and soil, the analyses were regulated with the addition of slopes obtained by interpolation values for each interval (t_3 slope, t_6 slope and t_{10} slope). The t-slopes were calculated from the initial temperature (t_0) to the final one, including all the internal steps (i.e., t_{10} slope was calculated from the t_0, t_3, t_6 and t_{10} values).

Three different datasets were hence created. The first in the laboratory method based on the active infrared thermography considered only the measurement of temperature variation as independent (observed) variable. The second laboratory one added the irradiation of soil samples as independent variable and it was based on active infrared thermometry. Finally, the in-field experiment was based on active infrared thermometry and it also considered as independent variables some meteorological parameters (i.e., air temperature and relative humidity, wind speed and irradiance at soil surface).

The SWC estimation in all the analysis, both in laboratory (i.e., thermographic and thermometric) and in the field (i.e., thermometric), was carried out by multivariate PLS regression analysis (Ulissi et al., 2011) on the basis of thermal data collected at the intervals t_3, t_3 slope, t_6, t_6 slope, t_{10} and t_{10} slope for the thermographic analysis and only at the interval t_{10} slope for both laboratory and in-field thermometric analysis taking a temperature reading every second. For these two last analyses only the t_{10} slope was considered because it performed better than the t_3 and t_6 ones. This is due to the presence in-field of the environmental variables producing noises that can be lowered with a longer acquisition.

The procedure of PLS (Wold et al., 2001; Menesatti et al., 2010) was elaborated using the PLS Toolbox in MATLAB V7.0 R14 (The Math Works, Natick, MA, USA) and included the following steps: (1) extraction of raw thermal data (X-block variables); (2) extraction of measured SWC (Y-block variables); (3) data fusion of the two dataset (Y and X-block) in one analysis dataset (AS); (4) the sample set partitioning based on joint x-y distances (SPXY) (Harrop Galvao et al., 2005; Ulissi et al., 2011) separation of the AS into two subsets, one (MS) for the model (85% of AS) and one (TS) for the external validation test (15% of AS); (6) application of different pre-processing algorithms (Table 4.2.1.2.3.1) to X-block and Y; (7) application of chemometric technique (PLS): modelling and testing; (8) calculation of efficiency parameter of prediction.

Table 4.2.1.2.3.1: List of the different X and Y pre-processing techniques applied in the analysis.

Label	Description
None	No pre-processing
Baseline	Baseline (Weighted Least Squares)
Abs	Takes the absolute value of the data
Autoscale	Centres columns to zero mean and scales to unit variance
Detrend	Remove a linear trend
Groupscale	Group/block scaling
Mean center	Center columns to have zero mean
Median centre	Centre columns to have zero median
Normalize	Normalization of the rows
SNV	Standard Normal Variate
Centering	Multiway Center

The predictive ability of the model is partially dependent on the number of Latent Vectors (LV) used and was assessed by the prediction efficiency parameters: Root Mean Square Error (RMSE), Standard Error of Prevision (SEP) and correlation coefficient (*r*) between observed and predicted values. Finally, we recorded the Ratio of Percentage Deviation (RPD), which is the ratio of the standard deviation of the laboratory measured (reference) data to the RMSE (Williams, 1987). It is the factor by which the prediction accuracy has been increased compared with using the mean of the original data. The model chosen was for the number of LV that yielded the highest *r*, minimum SEP for predicted and observed water content and maximum RPD.

In order to obtain more general and wide (i.e., mapping) estimations of soil water content characteristics in the in-field analysis a Partial Least Squares Discriminant Analysis (PLSDA) (Costa et al., 2010) was performed. This model considered three different classes of water content (low < 11%; 11% < medium < 14% and high > 14%) and calculated a prediction probability and a classification threshold for each class modelled. The samples from each class were subdivided in two subsets: (i) 75% of samples for the class modelling and validation; (ii) 25% of specimens for the independent test, optimally chosen with the Euclidean distances based on the algorithm of Kennard & Stone (1969) that selects objects without the a priori knowledge of a regression model (the hypothesis of a flat distribution of the data is preferable for a regression model). This analysis provided the percentage of correct classifications and the modelling efficiency in terms of sensitivity and specificity parameters where the first represents the percentage of the samples of a category accepted by the class model and the second the percentage of the samples of the categories different from the modelled one, rejected by the class model.

4.2.1.3 Results

4.2.1.3.1 Laboratory Results

4.2.1.3.1.1 Thermographic Results

Table 4.2.1.3.1.1.1 shows the results of PLS for the prediction of SWC through thermographic analysis for the three time intervals (t_3, t_6 and t_{10}) and for the slopes obtained by value interpolation for each interval (t_3 slope, t_6 slope and t_{10} slope) with maximum *r* and RPD (calculated to RMSE of test subset) and minimum SEP for the calculation of water content.

Table 4.2.1.3.1.1.1: Partial Least Squares (PLS) results for the prediction of soil water content (SWC) obtained with laboratory thermographic analysis for the three time intervals (t3, t6 and t10) and for the slopes obtained by values interpolation for each i interval (t3 slope, t6 slope and t10 slope). The table reports n° of Latent Vectors (LV); first and second pre-processing for the X-block and one for the Y-block; the correlation coefficient (r); the Ratio of Percentage Deviation (RPD); the Standard Error of Prevision (SEP) and the Root Mean Square Error (RMSE) for the model and test.

Parameters	t_3	t_3 slope	t_6	t_6 slope	t_{10}	t_{10} slope
MODEL (85%)						
n° LV	3	3	3	2	10	9
First pre-processing X-block	autoscale	none	autoscale	autoscale	autoscale	median center
Second pre-processing X-block	normalize	none	none	none	median center	none
Pre-processing Y-block	median center	autoscale	none	autoscale	none	median center
***r* (observed *vs.* predicted)**	0.3051	0.5765	0.6016	0.6113	0.7524	0.7756
RPD	1.0476	1.2209	1.209	1.2606	1.5133	1.5804
SEP	15.093	12.929	13.045	12.37	10.323	9.9083
RMSE	16.125	12.898	21.675	12.341	10.301	9.8858
TEST (15%)						
***r* (observed *vs.* predicted)**	0.2993	0.5198	0.5784	0.542	0.7227	0.7417
RPD	1.0322	1.1013	1.209	1.129	1.2163	1.4524
SEP	16.682	15.438	15.407	15.335	14.729	12.314
RMSE	19.444	15.286	27.478	16.258	26.281	15.512

4.2.1.3.1.2 Thermometric Results

Table 4.2.1.3.1.2.1 reports the results of PLS for the prediction of SWC through thermometric analysis only for the t_{10} slope interval, assessed with maximum *r* and RPD (i.e., calculated to RMSE of test subset) and minimum SEP for the calculation of water content. Figure 4.2.1.3.1.2.1 shows the regression between observed and predicted values relatively to the prediction for the prediction of SWC through thermometric analysis in the independent test for t_{10} slope.

Table 4.2.1.3.1.2.1: Results of Partial Least Squares (PLS) for the prediction of soil water content (SWC) obtained with laboratory thermometric analysis for the interval t10 slope. In the table are reported: n° of Latent Vectors (LV); first and second pre-processing for the X-block and one for the Y-block; the correlation coefficient (r); the Ratio of Percentage Deviation (RPD); the Standard Error of Prevision (SEP) and the Root Mean Square Error (RMSE) for the model and test.

Parameters	t10 slope
MODEL (85%)	
n° LV	4
First pre-processing X-block	autoscale
Second pre-processing X-block	none
Pre-processing Y-block	autoscale
***r* (observed *vs.* predicted)**	0.7095
RPD	1.4024
SEP	2.125
RMSE	2.1001
TEST (15%)	
***r* (observed *vs.* predicted)**	0.7634
RPD	1.2868
SEP	4.5316
RMSE	4.2138

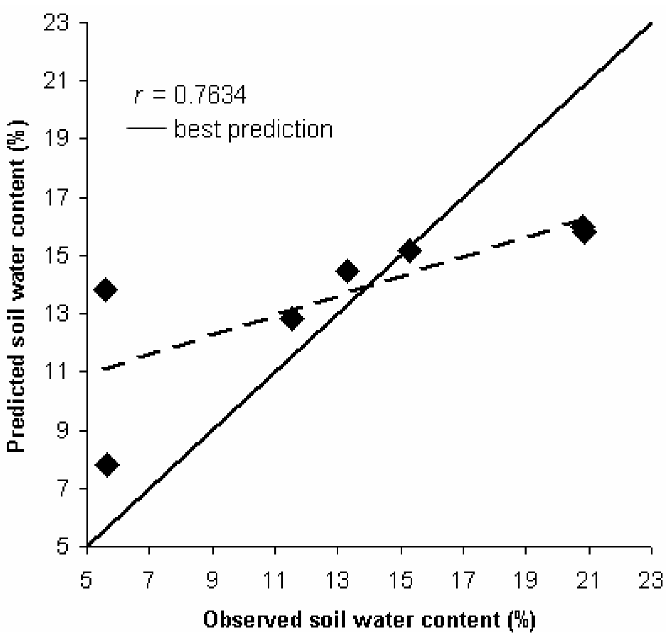

Figure 4.2.1.3.1.2.1: Regression between observed and predicted values of soil water content for the intervals t10 slope in the independent test for the thermometric analysis (i.e., 15% of whole sample dataset).

4.2.1.3.2 In-Field Results

Table 4.2.1.3.2.1 reports the results for the prediction of SWC through thermometric analysis performed in-field for the only interval t_{10} slope. Table 4.2.1.3.2.2 reports the results of the PLSDA for the prediction of SWC through thermometric analysis performed in-field for the only interval t_{10} slope considering three different classes of SWC (low < 11%; 11% < medium < 14% and high > 14%).

Table 4.2.1.3.2.1: Results of Partial Least Squares (PLS) for the prediction of SWC obtained with thermometric analysis performed in field for the interval t10 slope. In the table are reported: n° of Latent Vectors (LV); first and second pre-processing for the X-block and one for the Y-block; the correlation coefficient (r); the Ratio of Percentage Deviation (RPD); the Standard Error of Prevision (SEP) and the Root Mean Square Error (RMSE) for the model and test.

Parameters	t10 slope
MODEL (85%)	
n° LV	5
First pre-processing X-block	mean center
Second pre-processing X-block	baseline
Pre-processing Y-block	autoscale
***r* (observed *vs.* predicted)**	0.6383
RPD	1.2803
SEP	1.6924
RMSE	1.6726
TEST (15%)	
***r* (observed *vs.* predicted)**	0.6063
RPD	0.9742
SEP	3.6123
RMSE	3.3194

Table 4.2.1.3.2.2: Results of Partial Least Squares Discriminant Analysis (PLSDA) for the in-field prediction of SWC obtained with thermometric analysis for the interval t10 slope considering three different classes of soil water content (SWC) (low < 11%; 11% < medium < 14% and high > 14%). N is the number of samples; n° units (Y-block) is the number of units to be discriminated by the PLSDA; n° LV is the number of latent vectors. Random Probability (%) is the probability of random assignment of an individual into a unit.

Parameters	t10 slope
N (Low SWC < 11%)	13
N (11% < Medium SWC < 14%)	19
N (High SWC > 14%)	9
n° units (Y-block)	3
n° LV	6
% Cumulated Variance X-block	100
Mean Specificity (%)	89.033
Mean Sensitivity (%)	86.667
Random Probability (%)	33.333
Mean Classification Error (%)	12.143
Mean Correct Classification Model (%)	86.349
Mean Correct Classification Test (%)	88.889

4.2.1.4 Discussion and Conclusions

As reported by Bittelli (2011), SWC estimation is necessary for different applications, ranging from large-scale calibration of global-scale climate models to field monitoring in agricultural and horticultural systems. The proposed laboratory and in-field methods concern the development of non-destructive and rapid SWC estimations using active infrared thermography and thermometry in combination with multivariate statistical analysis (PLS). The main principle of these applications regards the measurement of the thermal infrared spectrum of electromagnetic radiation emitted by samples depending on their dynamic temperature variation achieved by heating soil (Maldague, 1994; Rahkonen & Jokela, 2003*)*. In this work the statistical modelling based on a variant of the heat dissipation method occurs efficacy in all the experimental analysis overcoming the limits of the heat dissipation sensors measuring directly the temperature dynamic variation of soil samples after heating.

Generally among the results shown by both laboratory and in-field applications, the best performing models were the t_{10} slope ones. The laboratory analysis showed that active infrared thermometry performed better than thermography, probably due to the variable represented by the irradiance present in the statistical model. This latter increased the correlation coefficient (*r*) in the independent test (0.7417 for thermography; 0.7634 for thermometry) but it especially decreased both SEP and RMSE (12.314 and 15.512 for thermography; 4.5316 and 4.2138 for thermometry). The RPD values remained instead similar (1.4524 for thermography and 1.2868 for thermometry).

Since the best performing laboratory model was thermometry, we have chosen to use only this methodology for the in-field applications in order to measure SWC from the dynamic variation of surface temperature after deep ploughing and primary tillage. Both ploughing and tillage were used only for practical reasons. This in-field measurement is influenced by soil-atmosphere interactions as reported by Campbell & Norman (1998). This makes the use of calibration curves unsuitable temperature to SWC as physical or empirical relationships which describe all the soil-atmosphere interactions. Moreover, for a correct infrared thermal measurement the estimation of emissivity is very important. As reported by Schmugge (1998) the rate of soil emissivity is a function of its texture and it is greater for lighter sandy and smaller for heavier clayey soils and it is reduced by surface features, such as roughness and vegetation cover. In this study, to overcome the heterogeneity of soil in terms of surface, temperature and water content we analyzed a great number of diverse non-factorial samples, mostly in the laboratory. Adapting the energy balance to the proposed study, this becomes dependent by agro-pedological and meteorological parameters such as air temperatures and humidity, SWC, irradiance, wind regimes, soil water potential and soil roughness making the system approachable in a statistic way instead of deterministic one. Thus, the estimation of SWC was developed by using a multivariate analysis by taking into consideration different soil thermal properties and meteorological parameters as input variables. This statistical approach provided a probabilistic framework for data analysis, as based on joint spatial and temporal dependence among observations (Castrignanò et al., 2003; Menesatti et al., 2010; Antonucci et al., 2011).

In particular, the proposed in-field method performed worse than the laboratory ones (r=0.6063 and RPD=0.9742), but reported very low prediction error values (SEP=3.6123 and RMSE=3.3194). In this case, the lower performance could be related to the measurement of thermo-hygrometer variables. In fact, the air temperature and relative humidity and wind speed were not punctually collected (i.e., we used a weather station). In order to obtain more general and wide (i.e., mapping) estimations of SWC in in-field analysis a PLSDA was performed. In this case three different classes of water content (low < 11%; 11% < medium < 14% and high > 14%) were considered and a prediction probability and a classification threshold were calculated for each class modelled. The results showed a higher percentage of the mean correct classification both in the model (86.349%) and in the test (88.889%) with respect to the PLS. This classificatory model using a multivariate statistical approach as shown above can discriminate among close classes of SWC. This proves as such a technique is capable of a fine discrimination with respect to a simple linear modelling approach. This in-field gives the opportunity to choose properly the best trafficability and soil workability. Therefore, relationships between soil water and environmental factors need to be studied over wider time- and spatial-scales (Owe et al., 1982). However, these could be implemented on vehicle-mounted systems to shorten sampling time and amount of soil needed. Moreover, it could be possible to obtain temperature mapping and consequently water content directly in field of any particular region of interest with fast response times, which is not presently possible with thermocouples or other temperature sensors (i.e., these can only measure spot data). In addition the repeatability of these measurements is high and it does not require an illumination source, unlike other systems (Vadivambal & Jayas, 2011).

Finally, both thermography and thermometry are of fast execution and could produce highly informative results if paired with a Geographic Information System (GIS). Also, as reported by Schmidhalter et al. (2008), these could be applied on site-specific management tasks, as required in precision farming to obtain detailed information about the heterogeneity of soil. Moreover, the proposed methodologies resulted very interesting for the limited time of exposure to the heat, needed to obtain results on dynamic temperature variation, making these implementable on commercial machine systems for very expeditious in-field applications.

4.2.1.5 Acknowledgements

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4.2.1.6 References

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5. Pre-harvest

The pre-harvest sector refers to all the agricultural activities that occur before crop products are sold (Womach, 2005). Pre-harvest production practices may seriously affect post-harvest returns. As reported by Crisosto et al. (1997) the maximum fruit quality for each cultivar can be achieved only by understanding the roles of pre-harvest factors (e.g., mineral nutrition, irrigation, crop load, fruit canopy position, market life potential, etc.). In facts, plants need a continuous supply of water for photosynthesis and transpiration. Damage can be caused by too much rain or irrigation, which can lead to decay; by too little water; and by irregular water supply, which can, for example, lead to growth cracks. Lack of plant food can affect the quality of fresh produce, causing stunted growth or discoloration of leaves, abnormal ripening and a range of other factors. Too much fertilizer can harm the development and post-harvest condition of produce. Good crop husbandry is important for reducing losses. Weeds compete with crops for nutrients and soil moisture. Decaying plant residues in the field are also a major loss factor (Kader, 2005).

As reported by López Camelo (2004) maturity is the harvest index most widely used in fruit. However, physiological maturity needs to be distinguished from commercial maturity. The former is reached when development is over. It may or may not be followed by the ripening process to achieve the commercial maturity required by the market. Every fruit shows one or more apparent signs when it reaches physiological maturity. Over-maturity or over-ripening is the stage that follows commercial maturity and occurs when the fruit softens and loses part of its characteristic taste and flavour (Fig. 5.1).

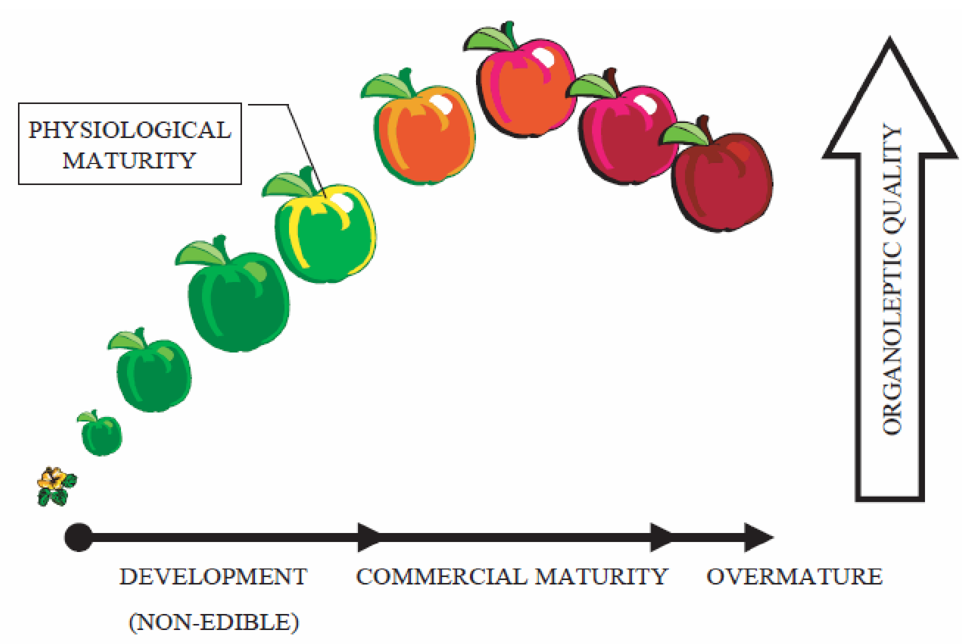

Figure 5.1: Organoleptic quality of a fruit in relationship to its ripening stage (from López Camelo, 2004).

Commercial maturity may or may not coincide with physiological maturity. Generally, there are two types of fruit: climacteric and non-climacteric. Climacteric fruit such as tomatoes and peaches are capable of generating ethylene, the hormone required for ripening even when detached from the mother plant. Non-climacteric fruit such as peppers and citrus obtain commercial maturity only on the plant. Climacteric fruit are autonomous from the ripening point of view and changes in taste, aroma, colour and texture are associated with a transitory respiratory peak and closely related to autocatalytic ethylene production. Climacteric fruit need to be harvested as early as possible for distant markets, but always after reaching their physiological maturity (López Camelo, 2004). The main criteria used for harvesting most fruit and vegetables are colour and the degree of development, or both. It is, however, common to combine these with other objective indices. These include, for example, firmness (apple, pear, stone fruit), tenderness (peas), starch content (apple, pear), soluble solid content (melons, kiwifruit), oil content (avocado), juiciness (citrus), sugar content/acidity ratio (citrus), aroma (some melons) (López Camelo, 2004).

5.1 Crop nutritional status estimation

The nutritional requirements of the crop are dependent on factors such as soil fertility, weather, planting age and crop load, all of which change over time. Therefore, the amount of nutrients the grower needs to provide the crop may also change over time. As the soil is the storehouse for nutrients, the best approach to meeting the nutritional requirements is to establish your crop in fertile, well drained soils with the appropriate soil pH. Once the crop is planted, routine evaluation of plant nutrient status and soil composition are essential to developing sustainable nutrient management practices.

The most common tools that fruit growers should utilize to evaluate the nutritional status of fruit crops in the pre-harvest activities are: visual symptoms, tissues chemical analysis and soil analysis.

The tissues chemical analysis can provide a more accurate understanding of nutrient status than visual diagnosis and can identify low nutrient levels before any significant crop impact occurs. Any direct determination on plant tissues seems more reliable to evaluate if the nutritional status of the crop is around, below or over the optimal one. For nitrogen estimation for example to define the optimal nutritional status of crops, Greenwood et al. (1990) developed the concept of critical N, which is the minimum N concentration in the plant required for maximum growth, and Lemaire & Gastal (1997) proposed the critical N dilution curve, since critical N concentration decreases with increasing biomass accumulation during the growth cycle. Hence, at any time of the growth cycle there is a critical N concentration below which the plant shows growth limitation and over which the plant shows luxury consumption (Greenwood et al., 1990; Lemaire & Gastal, 1997). Any species is supposed to have its own critical N dilution curve. Tei et al. (2002, 2003) found the curves for both total N and reduced N for either processing tomato or lettuce. Critical curves should be the absolute reference to evaluate the N nutritional status of any crop in any situation. Critical curves are calibrated for the whole plant biomass, or at least for the whole shoot biomass. However the leaf N content is often taken as indicator of the plant N nutritional status (Agostini et al., 2010).

Actually, 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 it is a major site of carbohydrate and mineral storage (Caloin & Yu, 1984).

The last common tool is represented by the soil analysis. Soil analysis is a valuable tool that can give information about the pH of the soil, organic matter content and can estimate the supply of nutrients in the soil available to plants. A soil analysis should always be done prior to planting as this is the best time to incorporate necessary soil amendments. Soil analyses aim to characterise the soil status or to predict its availability during the crop growth phase (Dachler, 2001). Several tests for soil N status for example (e.g., *Nmin* and *KNS*) (Wehrmann & Scharpf, 1986; Lorenz et al., 1989) are available that help guide fertilization, but their reliability depends of many variables and is not always satisfactory. In particular, their reliability depends on sampling procedure, since the N content in the soil is not homogeneous, and can vary during samples conservation while waiting analysis. Moreover, climatic and soil conditions (Wehrmann & Scharpf, 1986), as well as cultivation practices (Owen et al., 2003) can affect the magnitude of the soil nitrogen pool (i.e., the soil mineral N plus the N that will be released by soil organic matter mineralization within the growing season) available to roots, so that actual plant N nutritional status might result different from that expected on the basis of soil N assessment.

Generally these methods are punctual, destructive, expensive or time-consuming. In fact, these procedures are time consuming for most diagnostic situations in the field (Lemaire, 2007).

For N evaluation, quick tests like sap test or chlorophyll readings have been developed and now are more and more used (Simonne & Hochmuth, 2006; Farneselli et al., 2007, 2008). The sap test measures the N-NO_3 concentration in xylem and phloem sap plus the apoplastic, citosolic and vacuolar water on the leaves, thus it results a direct measure of current N supply. Once absorbed by roots, nitrogen is transported to the leaves where is transformed and incorporated into living material. Thus, nitrate concentration in the aerial part of the plant provides a good indication of the adequacy of N applied to the crop. In particular, nitrate in the leaf petioles seems to give the best indication of crop N nutritional status because is more sensitive to fluctuations in N availability than the sap extracted by leaf blade. Nitrate content on sap can be measured by different tools and in general it results high correlated to the conventional laboratory analysis (Hartz et al., 2000). Other optical-based quick nitrogen tests are the chlorophyll meter readings, such as the SPAD (Minolta L.t.d) readings. These quick tests helped to implement good practices in vegetable N management (Westwerld et al., 2003). Tremblay et al. (2007), in their guide to vegetable nitrogen fertilisation, give a very good judgement on the use of these field devices, but consider them as a complement to more conventional soil analysis. Others (Schroder et al., 2000; Neukirchen & Lammel, 2002) judged such methods, if calibrated according to varieties and environment, so precise to be able to fully supply laboratory determination for plant and soil.

The rapid and non-destructive detection of the nutritional status in terms of maturity, in pre-harvest activities could be an important action to limit the loss of quality of the final product. In fact to estimate the degree of maturity before harvest by the foliar analysis using a VIS-NIR portable spectrophotometer for example, could guide both fertilizer plans, limiting waste, and the sudden or late fruit harvest

5.1.1 Why monitor crop nutritional status?

These rapid and non-destructive methods are essential if a rapid crop nutritional status evaluation is required, especially to adjust the nutritional recommendation rate in a dynamic pre-harvest nutritional management.

For example intensive fertilization can cause environmental negative side-effects and health problems (Ramos et al., 2002). Nitrate pollution causes the well known eutrophication of surface waters and the contamination of groundwaters, with health risks related to meta-emoglominemia in young animals and children (Barret et al., 1998; European Commission, 1998). Finally, nitrate accumulation in edible portion of over fertilized leafy vegetables would involve the production in human bodies of carcinogenic compound like nitrosamines (Hartz, 2003).

All this negative side effect can be limited by a rational fertilization technique. Fertilization is well conceived and managed when it can provide an adequate crop nutrition avoiding both deficiencies and excesses (Battilani, 2001). Deficiencies would limit crop yield and farmer’s income; excess would amplify negative side effects in front of risible or no increase of yield and profit. Hence, the fertilization rate and the timing and manner of nutrient delivery should be conceived to meet crop needs at any growth stage.

Localized, starter, split fertilization are the strategies developed to guaranty a proper and flexible N delivery to crops (Batal et al., 1994). With this regard, the best example of localized and split fertilization is fertigation that is the application of nutrients with irrigation water by drip systems (Battilani et al., 2003). Fertigation allows to split the rate according to crop requirement at any growth phase and to localize the fertilizer close to roots (Singandhupe et al., 2003), and as a consequence it increases water and N use efficiency and reduces the risks of N leaching below the root zone (Farneselli et al., 2007; 2008). A flexible in-season adjustment of N fertilization requires knowledge about crop specific nutrient requirements throughout the growth cycle (Tei et al., 2002) and a continuous monitoring of crop nutritional status by precise, rapid and cheap measurement. These last aspects become essential and are the subject of many recent studies.

5.2 Case studies

For the pre-harvest phase, two case studies were conducted (Fig. 1.1). All these case studies were published in international peer-reviewed journals with impact factor.

The first proposed the use of visible-near infrared (VIS-NIR) portable spectrophotometer to evaluate citrus crops nutritional status represented by various macroelements (i.e., N, P, K, Ca, Mg, Fe, Zn and Mn) through foliar analysis. The objective of the second case study evaluated the possibility and the accuracy of the estimation of tomato leaf nitrogen concentration performed through the same system, in comparison with chemical standard analyses, chlorophyll meter readings and $N\text{-}NO_3$ concentration in petiole sap (Fig. 1.1).

5.2.1 Menesatti P, Antonucci F, Pallottino F, Roccuzzo G, Allegra M, Stagno F & Intrigliolo F (2010) Estimation of plant nutritional status by VIS-NIR spectrophotometric analysis on orange leaves [Citrus sinensis (L) Osbeck cv Tarocco]. Biosystem Engineering, 105, 448-454. (IF2010=1,241)

Abstract

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 (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 P with standard error of prevision ranging from 0.01 for P to 12.418 for Fe.

Keywords: Citrus nutritional status, spectrophotometry, VIS-NIR, orange leaves.

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

5.2.1.1 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 et al., 1973a; Embleton et al., 1996; Koo, 1989; Legaz-Paredes & Primo-Millo, 1988), whilst minimising the amount of chemical fertilisers applied, to reduce the risks of environmental impact (Alva et al., 2003a Davies, F.S. 1997).

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 et al., 1973a; Hanlon et al., 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 et al., 1973b). Analytical evaluations are performed more frequently, and with different aims, directly on the fruit as reported by many research (Steuer et al., 2001; McGlone et al., 2003; Gomez et al., 2006; Cayuela, 2008).

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 (Obreza et al., 1992; Ferguson et al., 1995; Intrigliolo et al., 1998). 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 et al. (1973b) reported the leaf analysis standards for mature, bearing orange trees based on 5 to 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 (Obreza et al., 1992; Intrigliolo et al., 1999).

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 (kind and strength) and temperatures that should be taken into account during data processing as suggested by Ventura et al. (1998). Walsh et al. (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 (Temma et al., 2002; Hernandez Sanchez et al., 2003; Saranwong et al., 2003a, b; Miller & Zude-Sasse, 2004; Zude et al., 2006). Another well known and established techniques that must be considered while exploring the nutritional status of a plot of land, that is based on the SPAD Chlorophyll Meter (Piekielek et al., 1995; Lee et al., 1999; Read et al., 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 proposed and its results compared with results from standard chemical analyses.

5.2.1.2 Materials and Methods

5.2.1.2.1 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 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 field chosen, in a randomised block design, the trees received different nitrogen treatments to be sure of 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 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. The remaining 20 leaves, were analyzed at the CRA-ING Laboratory using spectrophotometric techniques. The acquisition of the raw spectral curve through the spectrophotometer took about 2 s for each leaf.

5.2.1.2.2 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 hr, iv) ground and v) dried at 105 °C for 4 hr. 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 hr. 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.

5.2.1.2.3 Spectrophotometric analysis

For the VIS-NIR measurements, a (portable) single channel spectrophotometer was used (Fig. 5.2.1.2.3.1). 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 20W halogen lamp and an optical fibre bundle consisting of approx. 30 quartz glass fibres; 2) an optical entrance with input round: 70 µm x 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.

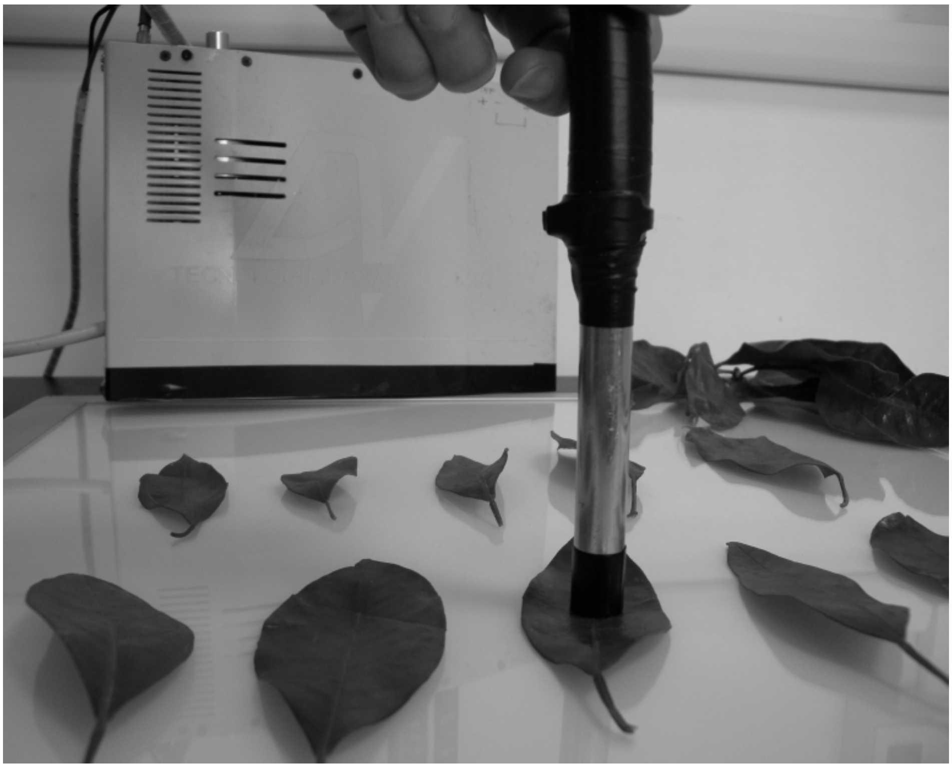

Figure 5.2.1.2.3.1: Performing VIS-NIR spectral measurement on citrus leaves.

The Hamamatsu spectrograph has 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.

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. 5.2.1.2.3.1). 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.

5.2.1.2.4 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 Partial Least Squares (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 et al., 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 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 50,000 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.

5.2.1.3 Results

Table 5.2.1.3.1 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 5.2.1.3.1: Descriptive statistical values of elements measured on thirty citrus leaves for each treatments 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

Table 5.2.1.3.2 shows the values and results of the PLS prediction of the SINGLE chemical values predicted.

Table 5.2.1.3.2: 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	normalize	baseline	osc	baseline	gls weighting	normalize	baseline
Pre-processing Y-Block	median center	mean center	mean center	mean center	median center	median center	median center	mean center
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

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. 5.2.1.3.1). 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.

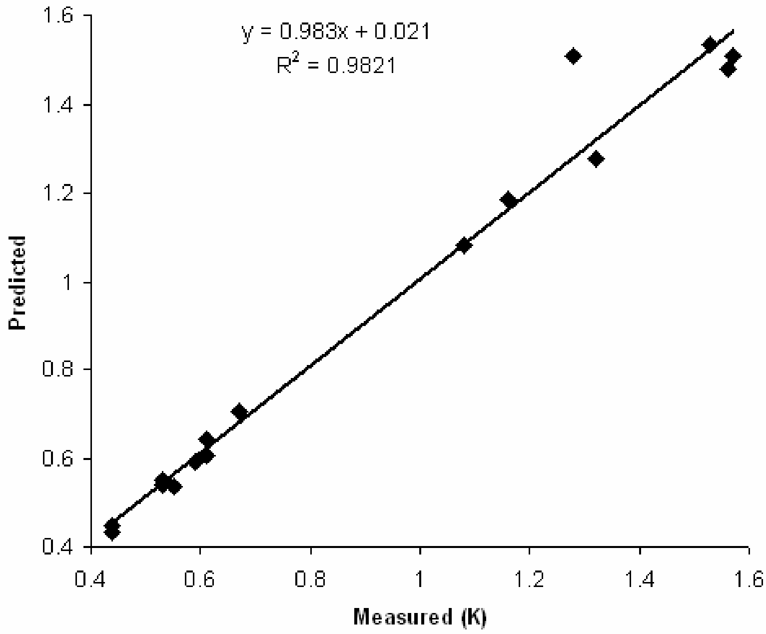

Figure 5.2.1.3.1: Correlation between measured and predicted values of K.

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 5.2.1.3.2). The model of this parameter also had low values of SEP (0.039) and RMSE (0.039). Fig. 5.2.1.3.2 shows the correlation between predicted values and the observed chemical values (N) with a high value of R^2 (0.8265).

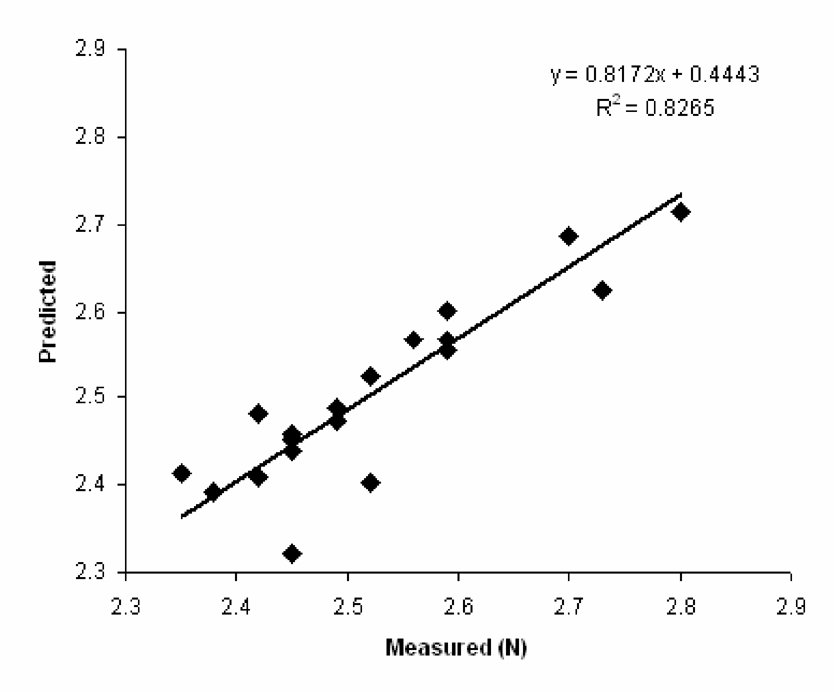

Figure 5.2.1.3.2: Correlation between measured and predicted values of N.

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. 5.2.1.3.3).

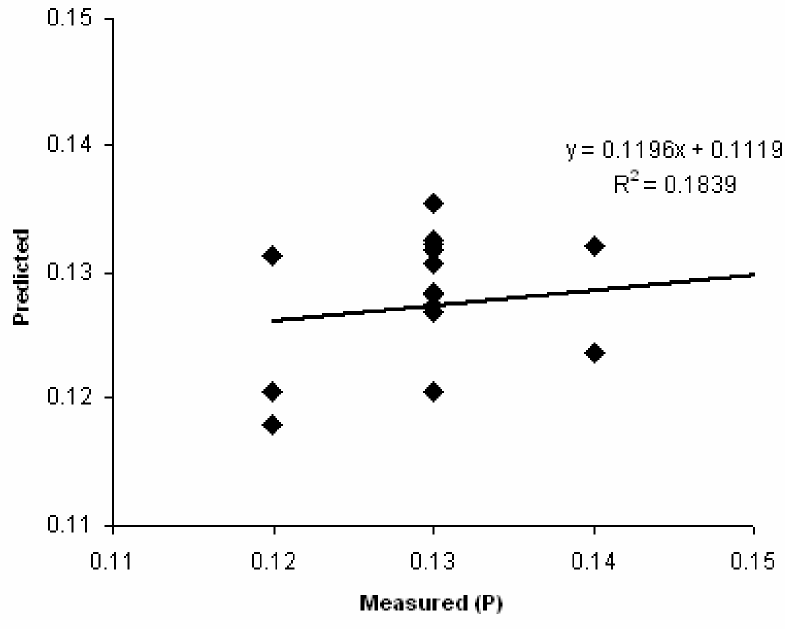

Figure 5.2.1.3.3: Correlation between measured and predicted values of P.

Table 5.2.1.3.3 shows different results for the ALL elements predicted. Indeed the analysed elements showed *r* values in a range 0.883 (Mg) to 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, normalize pre-processing of the X-block and auto scaling for the Y-block.

Table 5.2.1.3.3: 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

5.2.1.4 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 et al., 2003b). 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 a 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 et. al, 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.

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 concentration in the leaves as previously reported in the literature (Embleton, 1973a, b).

Many researchers successfully used spectral systems to evaluate the N status of different crops (Sui et al., 1998; Tumbo et al., 2002a, b and c). 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 5.2.1.3.3), showed inferior performance compared with the SINGLE models (Table 5.2.1.3.2), it could be an interesting application particularly if rapid measurements are needed.

5.2.1.5 References

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Abstract

Nitrogen concentration in plants is normally determined by expensive and time consuming chemical analyses. As an alternative, chlorophyll meter readings and $N\text{-}NO_3$ concentration determination in petiole sap were proposed, but these assays are not always satisfactory. Spectral reflectance values of tomato leaves obtained by visible-near infrared spectrophotometry are reported to be a powerful tool for the diagnosis of plant nutritional status. The aim of the study was to evaluate the possibility and the accuracy of the estimation of tomato leaf nitrogen concentration performed through a rapid, portable and non-destructive system, in comparison with chemical standard analyses, chlorophyll meter readings and $N\text{-}NO_3$ concentration in petiole sap. Mean reflectance leaf values were compared to each reference chemical value by partial least squares chemometric multivariate methods. The correlation between predicted values from spectral reflectance analysis and the observed chemical values showed in the independent test highly significant correlation coefficient (r=0.94). The utilization of the proposed system, increasing efficiency, allows better knowledge of nutritional status of tomato plants, with more detailed and sharp information and on wider areas. More detailed information both in space and time is an essential tool to increase and stabilize crop quality levels and to optimize the nutrient use efficiency.

Keywords: spectrophotometry; VIS-NIR; tomato leaf; non-destructive; SPAD chlorophyll meter readings; SAP test; leaf analysis; nutritional status; chemometry.

5.2.2.1 Introduction

There is an increasing effort to optimize nitrogen (N) fertilization and to improve crop N use efficiency in order to achieve high yields and limit environmental side effects related to N leaching (Agostini et al., 2010). This can be done by a fine-tuning of fertilization rate and a dynamic N management at each growth stage according to the nutritional status periodically monitored (Farneselli et al., 2008). This is the case of fertigated vegetables, such as processing tomatoes, where the fertilizer rate can be split and adjusted throughout the whole growing cycle by means of a drip irrigation system (Singandhupe et al., 2003).

Nevertheless, standard laboratory analysis of N concentration in the above-ground biomass are expensive and time consuming, especially if a rapid crop N status evaluation is required for in-season decision making procedures (Lemaire, 2007). For this reason, quick and practical tests have been proposed, some of which are already spread among growers. Opto-electronic based techniques can strongly help to reach the previously mentioned goals, thanks to easiness of use and low costs. Two of the most common and simple of these are: the chlorophyll meter readings (e.g., SPAD-502, Minolta) and the measurements of N-NO_3 concentration in petiole sap (SAP test).

The N nutritional state in plants may be determined indirectly by the chlorophyll concentration present in the leaves, as it is directly correlated to their N concentration (Sandoval-Villa et al., 2002). The SPAD method measures the light transmittance through leaves and is based on this correlation. It has been tested with good results in several arable and vegetable crops and also in tomato (Gianquinto et al., 2006; Farneselli et al., 2008). Nevertheless, the N prediction performance of the SPAD is variable, being affected by several factors such as cultivar, environmental conditions, plant growth stage, disease and pests (Gianquinto et al., 2006). In addition, Monje & Bugbee (1992) found that data from SPAD were closely correlated with destructive measurements of chlorophyll for leaves with chlorophyll concentrations ranging from 100 to 600 mg m^{-2}, but consistently overestimated chlorophyll outside this range.

The SAP test can be measured by different tools generally correlated to the conventional laboratory analysis (Coulombe et al., 1999). The most commons are: Merkoquant test strips, which react to the N-NO_3 concentration by producing a color, the intensity of which varies directly with the concentration; an ion-specific electrode, as Horiba-Cardy Meter, which reads directly the N-NO_3 concentration in the SAP. Several plant SAP quick test kits have been calibrated for N in many arable and vegetable crops and also in tomato (Farneselli et al., 2008). The N prediction performance by SAP test is variable being affected by many cultivar and agronomical factors (Farneselli et al., 2008), like SPAD. In tomato cultivation, it has been found to be in agreement with the critical-N curve method (Tei et al., 2002) for the most critical period of the fertilization management (Jimenez et al., 2006).

Both SPAD and SAP test however seem to better indicate N deficiencies than excesses, therefore their use for reducing over-fertilization would be not efficacious (Hartz, 2003). For these reasons it is very important to consider the critical N concentration, which is the minimum in the plant required for maximum growth (Lemaire & Gastal, 1997). Tei et al. (2002) proposed a critical N dilution curve for processing tomato which can represent the reference to evaluate if the crop is at sub-optimal (< 3.72%), optimal (between 3.72% and 4.81%) or luxury (between 4.81% and 5.2%) consumption at any time of the cycle.

In this study we propose a more complex and efficient opto-electronic method for the evaluation of N nutritional status in tomato leaves. It refers to the utilization of a visible-near infrared (VIS-NIR) portable spectrophotometer representing a rapid, non-destructive, cost-effective technique (Menesatti et al., 2010). The aim of this study was to evaluate the feasibility and accuracy of this method as compared to SPAD readings and SAP test and to reference standard chemical analysis.

5.2.2.2 Experimental Section

5.2.2.2.1 Data Collection

This study was carried out in 2008 at the Experimental Station of the Department of Agricultural and Environmental Sciences, in Papiano (Tiber Valley, Perugia province, Central Italy, 43°N, elev. 165 m) on a clay-loam soil. Processing tomato (*Lycopersicon esculentum* Mill., cv. PS1296) was grown in the field according to a randomized block design with three replicates where thirteen fertilisation treatments were compared, differing for application technique, N form and rate. Five of them were represented by green manures grown in the previous fall-winter and incorporated in early spring: green manures were hairy vetch (*Vicia villosa* Roth.) and barley (*Hordeum vulgare* L.) cultivated as monocultures at full sowing density (200 seeds m^{-2} for vetch and 400 seeds m^{-2} for barley) and as intercrops obtained by using a fraction of the full sowing density for each species according to a substitutional approach, namely 75% of vetch + 25% of barley, 50% + 50% and 25% + 75%. The total N supplied by green manures varied from 252 kg ha^{-1} to 183, 167, 160 and 154 as the proportion of vetch decreased from 100% (pure vetch) to 0% (pure barley). Previous experiments have shown that actual N release in the soil from green manures above is much different in time, with pure barley causing N deficiency during early stages of the following cash crop (Benincasa et al., 2008, 2010). The other eight treatments included: broadcast all-at-once application of two organic fertilisers (poultry manure and by-product from leather factory, both at 100 kg N ha^{-1}); localised and split fertigation with one organic and one mineral fertiliser at 2 different rates (100 and 200 kg N ha^{-1}); two unfertilised controls, one with tomato in plots where no crop was grown in autumn-winter and one with tomato in plots where barley was grown and then mown and removed from the field before tomato transplanting in order to cause the maximum depletion of soil available N.

The supply of P and K was adjusted taking into account the amount supplied with organic fertilizers, in order to obtain the same rate for all N fertilization treatments (75 kg ha^{-1} of P_2O_5 and 75 kg ha^{-1} of K_2O). The same irrigation volume was applied in a two-times-per-week irrigation schedule for all treatments, according to potential crop evapotranspiration. The N nutritional status of the crop was evaluated on three sampling periods (s.p.), 25 June (37 Days After Transplanting, DAT), 9 July (51 DAT), and 23 July (65 DAT), in coincidence with plant samplings for growth analysis (1^{st} s.p., 2^{nd} s.p. and 3^{rd} s.p. respectively). Each sampling period corresponds to a specific phenologic stage of the tomato plantation: 1^{st} vegetative growth, 2^{nd} early flower fruit and 3^{rd} fruit bulking.

At each sampling date eight plants per plot were harvested. The SPAD readings were taken on the apical leaflet blade of the youngest fully expanded leaf of those plants; the petioles of the same leaves were then collected and SAP nitrate concentration was measured by an ion-specific electrode meter. Then the eight leaflets above, plus other 16 leaflets detached from other young fully expanded leaves of the same eight plants per plot, were stored at 5°C in plastic envelopes in the dark and carried to the CRA-ING laboratory (Lat. 42°06’11.00” N, Long. 12°37’40.81” E) where VIS-NIR measurements were performed within three hours. Thirteen-fifteen leaves were spectrally measured two times, randomly acquiring nearly 3,100 full spectra. The N concentrations of the leaflets used for VIS-NIR measurements and of the whole above-ground plant subsamples were then measured by analysis of dry matter; an automatic analyser (FlowSys, Systea, Italy) was used to measure organic-N concentrations on digests prepared according to Isaac & Johnson (1976).

5.2.2.2.2 SPAD Analysis and SAP Test

The SPAD readings were taken from the apical leaflet of the youngest fully expanded leaf; the petioles of the same leaves were then collected and SAP nitrate concentration was measured by an ion-specific electrode meter (Cardy, Spectrum Technologies, Inc., Plainfield, IL, USA). The N concentration of the leaves and of the whole plant were then measured by analysis of dry matter; an automatic analyser (FlowSys, Systea, Italy) was used to measure organic-N concentrations on digests prepared according to Isaac & Johnson (1976).

5.2.2.2.3 Spectrophotometric Analysis

For the VIS-NIR measurements, a (portable) single channel spectrophotometer was used. The system is composed of five parts: (1) a Hamamatsu S 3904 256Q spectrograph in a special housing; a customized illumination system realized by a 20 W halogen lamp and an optical fiber bundle consisting of approx. 30 quartz glass; (2) an optical entrance with input round: 70 µm × 2,500 µm and diameter 0.5 mm NA=0.22 mounted in SubMiniature version A-coupling; (3) specific probes with quartz optical fiber of connection; (4) a transmission device for transmitted or absorbed light for thin solids or liquid with variable optical length; (5) a notebook equipped with specific software to acquire, calibrate and elaborate spectral data. The Hamamatsu spectrograph has the following characteristics: grating: flat-field, 366 line/mm (centre); spectral range: 310–1,100 nm; wavelength accuracy absolute: 0.3 nm; temperature-induced drift: < 0.02 nm/K; resolution (Rayleigh-criterion): DlRayleigh » 10 nm; sensitivity: » 1,013 Counts/Ws (with 14-Bit-conversion); straylight: < 0.8% with halogen lamp and 16 bit A/D converter.

For spectral acquisition, the 'pen' probe was used to measure the spectral reflectance response on each single leaf (spot area ≈ 10 mm^2). On each leaf two spot areas were acquired with the pen probe in the same areas used for SPAD and SAP test analysis. The reflectance measure is referred to the light percentage that is reflected by the material and acquired by an optical quartz fiber (0.7 mm in diameter) fixed at 45° inside a circular aperture of 4 mm in diameter, in relation with a white reference (100% of the signal available). The material surface due to its softness was able to include the entire circular aperture avoiding any external light interference. The spectral measurements were performed in laboratory considering a white calibration (lower value with respect to the external light), the instrumental integration time (light acquisition time) and subtracting the background noise (variable in function of the instrument temperature). A very low signal/noise ratio was observed in the beginning and at the end of the spectral range, affecting the accuracy measurements, so only the spectrum in the range 400-800 nm were take into account for the analysis. All spectral values were expressed in terms of relative reflectance. To remove drift effect for each group 12-15 leaves were chosen at random. After 30-35 spectral measurements a new white calibration was performed.

5.2.2.2.4 Chemometric Analysis of Spectral Data

Mean reflectance values of all leaves considering together all treatments, were compared to each reference chemical value by chemometric multivariate methods (Partial Least Squares, PLS). The procedure includes the following steps (Figure 5.2.2.2.4.1): (1) extraction of raw spectra dataset, to be used as X-block variables; (2) X-block variables selection; (3) creation of measured values dataset to be used as reference or response variable (Y-block); (4) data fusion of the two dataset (X- and Y-block) in one analysis dataset; (5) SPXY (sample set partitioning based on joint X- and Y-blocks) (Harrop Galvao et al., 2005) partitioning of the dataset into two subsets, one for the model (85% of whole dataset) and one for the external validation test (15% of whole dataset) (i.e., 85% of total samples were used to calibrate model and 15% were reserved for external validation). With respect to the random partitioning method, widely used in literature, SPXY approach, assigning equal importance to the samples distribution within both X- and Y-blocks, returns more objective and replicable results and could found analytical applications involving complex matrices, in which the composition variability of real samples cannot be easily reproduced by optimized experimental designs; (6) application of different pre-processing algorithms to X-block and Y; (7) application of chemometric technique (PLS): modelling and testing; (8) calculation of efficiency parameter of prediction.

All the spectral variables in the range 400–800 nm were selected to obtain four different X-block datasets: (i) Whole dataset (W; 125 vars; 400–811 nm); (ii) Restricted dataset 1 (R1; using the first 92 vars; 400–694 nm); (iii) Restricted dataset 2 (R2; 46 vars; 400–694 nm) represented by the mean values of each single consecutive pair of steps; (iv) Restricted dataset 3 (R3; 62 vars; 496–694 nm).

To divide the dataset into model (calibration and validation) and test sub-sets, for multivariate PLS analysis, the SPXY method (Harrop Galvao et al., 2005) was used. This method employs a partitioning algorithm that takes into account the variability in both x- and y-spaces. To obtain the best prediction test, different X and Y pre-processing techniques were applied (Table 5.2.2.2.4.1), from the simpler (none, Log 1/R, diff1, mean centre, autoscale, median centre, baseline) to the more specific for spectral data (Savitsky Golay, Multiple Scatter Correction, Orthogonal Signal correction). Pre-processing for X block was applied both as single pass then as double considering all possible combinations (i.e., Log 1/R + autoscale).

Prediction of nitrogen leaves concentration was performed by PLS regression model, using PLS Toolbox in MATLAB V7.0 (The Math Works, Natick, USA). PLS is a soft-modelling method (Wold et al., 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-block), after pre-processing treatments. The model determines the minimum set of the n estimation variables (LV, latent variables) by a recursive process. These variables could be represented in a n-dimensional space and they are used by PLS to calculate the best regression matrix between the X and the Y. The calibration models were also validated using full cross-validation, Venetian blind (Matlab rel. 7.1, PLSToolbox Eigenvector rel. 4.0). The model includes a calibration phase and a validation phase and for both phases it calculates the residual errors (Root mean square error in calibration

RMSEC and validation RMSECV). Modelling methods are subjected to over-fitting: this occurs when a model is excessively complex, such as having too many parameters (LV) relative to the number of observations. Therefore, in order to avoid the over-fitting is necessary to choose the model in order to optimize the number of LV in relation with efficiency parameters.

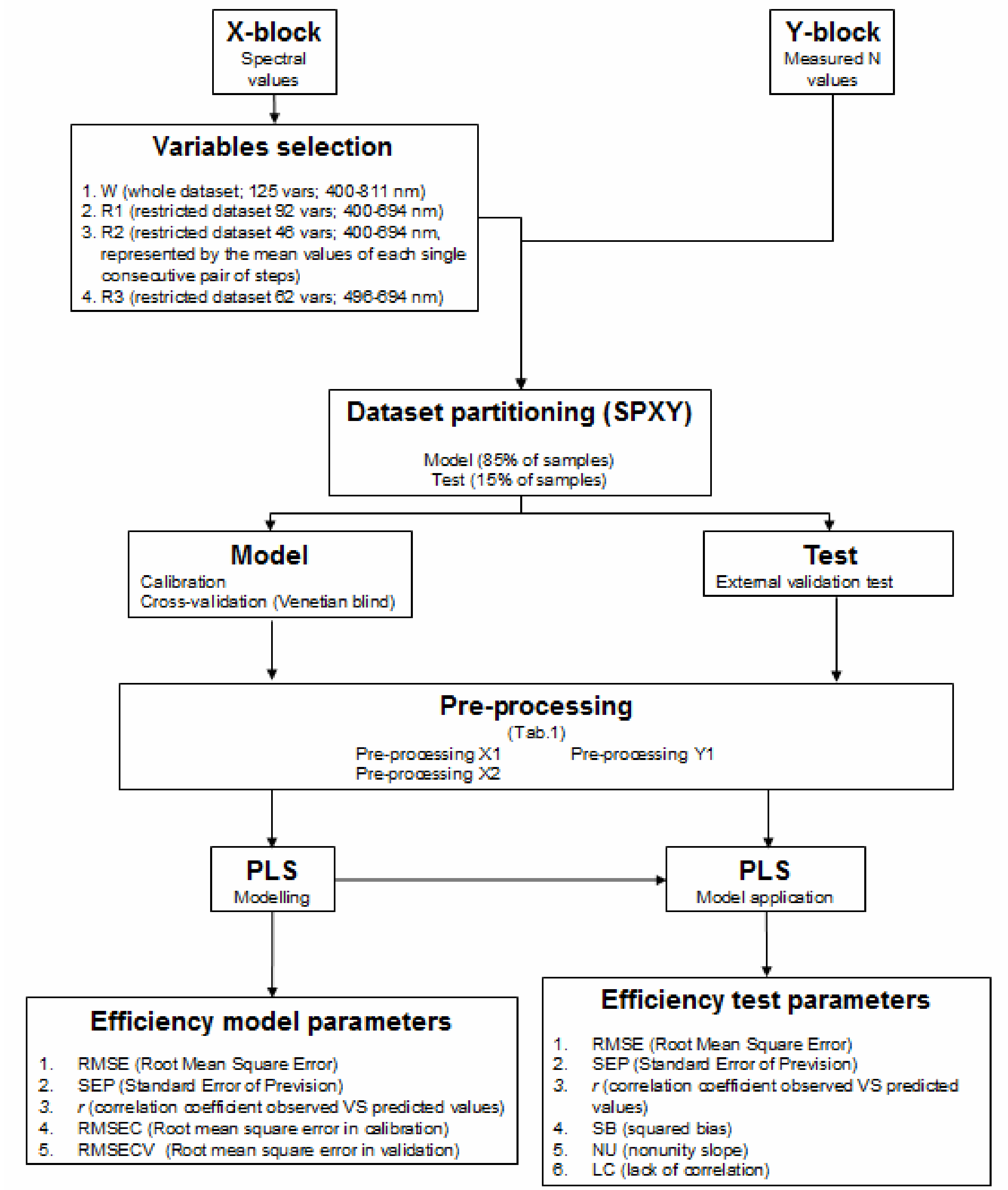

Figure 5.2.2.2.4.1: Spectral data chemometric analysis procedure.

Table 5.2.2.2.4.1: List of the different X and Y pre-processing techniques applied in the analysis.

Label	Description
None	No pre-processing
Log 1/R	Transformation of reflectance in absorbance following log(1/R) formula
Diff1	differences between adjacent variables (approximate derivatives)
Log10	Log 10
Logdecay	Log Decay Scaling
Baseline	Baseline (Weighted Least Squares)
Abs	Takes the absolute value of the data
Autoscale	Centres columns to zero mean and scales to unit variance
Detrend	Remove a linear trend
GLS Weighting	Generalized Least Squares Weighting
Groupscale	Group/block scaling
Mean centre	Centre columns to have zero mean
MSC (mean)	Multiplicative scatter correction with offset, the mean is the reference spectrum
Median centre	Centre columns to have zero median
Normalize	Normalization of the rows
Osc	Orthogonal Signal Correction
SG	Savitsky-Golay smoothing and derivatives
SNV	Standard Normal Variate
Centering	Multiway Center
Scaling	Multiway Scale
sqmnsc	Scale each variable by the square root of its mean

5.2.2.2.5 Regression Analysis on SPAD and SAP Data

Different monovariate regressions (linear, power, exponential and logarithmic) were calculated between the petiole nitrate concentration measured by the SAP and the total plant and leaf N concentration measured by laboratory analysis. The same analyses were also determined between the chlorophyll concentration assessed by SPAD analysis and both the total plant and leaf N concentration measured by the lab analysis.

The linear correlations between the N concentration chemically measured of the three different sampling periods (s.p. 1st, 2nd and 3rd) and the N concentration measured through SPAD analysis were calculated. To calculate the prediction efficiency, as in chemometric analysis, the whole dataset was divided into model (calibration and validation) and test sub-sets by means of the SPXY method (Harrop Galvao et al., 2005).

5.2.2.2.6 Predictive Accuracy of Models

The predictions obtained from the SPAD, SAP and PLS models in external validation subset were compared through linear regression analysis with the observed values.

Different accuracy parameters were extracted such as RMSE (Root Mean Square Error), SEP (Standard Error of Prediction) and correlation coefficient (*r*). The *r* was taken into consideration for distinguishing systematic errors and studying the correlation between the reference and predicted values. Generally, a good model should have high correlation coefficients *r*, low RMSE and SEP.

Others three parameters were calculated referring to Gauch et al. (2003): squared bias (SB), nonunity slope (NU) and lack of correlation (LC). In formulae (1, 2 and 3) they are defined as follow:

$$SB = \left(\overline{X} - \overline{Y}\right)^2 \tag{1}$$

$$NU = (1-b)^2 \times \left(\sum (x - \overline{X})^2 / N\right) \tag{2}$$

$$LC = \left(1 - r^2\right) \times \left(\sum (y - \overline{Y})^2 / N\right) \tag{3}$$

where x are the model-based predicted and y the measured values respectively, $\overline{X}$ and $\overline{Y}$ their mean, N the number of observations in the validation test, b is the slope and r^2 the square of the correlation.

In a perfect prediction, i.e., in the 1:1 line of equality Y=X, SB and LC should be equal to 0, while NU > 0 for b ≠ 1. In the accuracy analysis, SB is a good indicator of translation, NU of the rotation and LC of the scattering of the correlation line (Gauch et al., 2003).

5.2.2.3 Results

Figure 5.2.2.3.1 shows the cumulated nitrogen concentration frequency chemically measured in relation to the three different sampling periods (s.p. 1st, 2nd and 3rd).

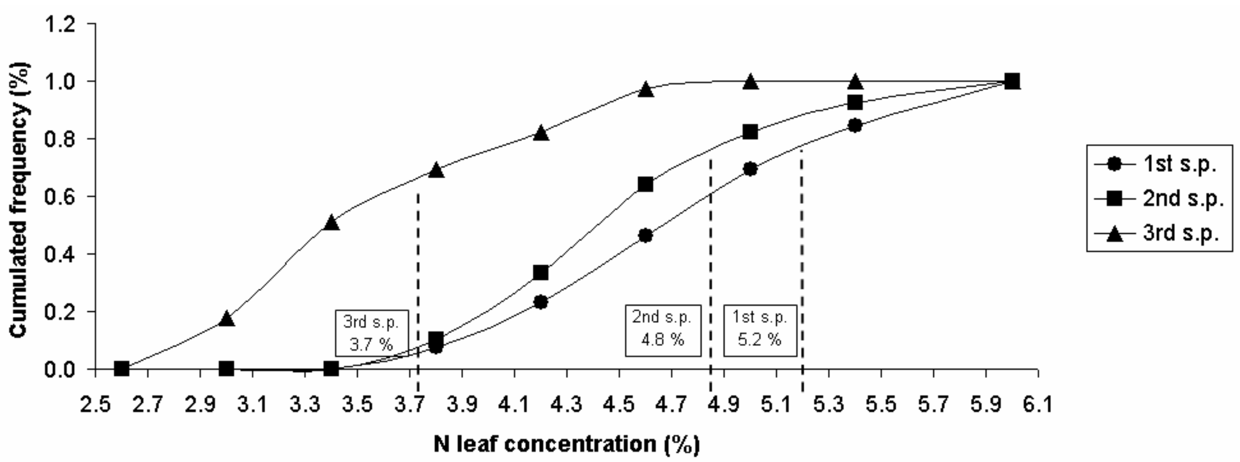

Figure 5.2.2.3.1: Cumulated nitrogen concentration frequency chemically measured in relation to the three different sampling periods (s.p. 1st, 2nd and 3rd) and the three thresholds (vertical lines) extracted by the critical-N curve proposed by Tei et al. (2002).

In the figure, the three thresholds extracted by the critical-N curve proposed by Tei et al. (2002) are also reported. These thresholds indicate the optimum N concentration of the plants depending on the phenologic stage of the tomato plantation. Approximately 80% of the samples were below the critical threshold of N leaf concentration during the first two sampling period, and ~65% were below the threshold for the 3rd sampling period.

The *r* values of the linear correlations between the N concentration chemically measured divided in the three different sampling periods (s.p. 1st, 2nd and 3rd) and the N concentration measured through SPAD analysis resulted very low: 0.5, 0.2 and 0.3 respectively.

Table 5.2.2.3.1 indicates the results of the linear regression of the model and test performed on the values of SPAD and SAP. The coefficient of correlation (*r*) of the test is equal to 0.56 in both SPAD and SAP test. The SEP and RMSE values are slightly lower for the SPAD analysis (0.72 and 0.64 respectively). Table 2 reports also the squared bias (SB), the nonunity slope (NU) and the lack of correlation (LC) for both SPAD and SAP test. These values are respectively equal to: SB=0.12 and 0.12; NU=0.0003 and 0.05; LC=0.48 and 0.48.

Table 5.2.2.3.1: Results of linear regression prediction of N concentration in tomato leaves from SPAD (chlorophyll meter readings) and SAP (measurements of N-NO_3 concentration in petiole) analysis. Efficiency parameters reported: correlation coefficient (*r*), Standard Error of Prediction (SEP), Root Mean Square Error (RMSE), Squared bias (SB), nonunity (NU) and lack of correlation (LC).

Parameter	SPAD	SAP
MODEL (85%)		
n° samples	100	100
***r* (measured *vs.* predicted)**	0.5383	0.5638
SEP	0.6260	0.6135
RMSE	0.3918	0.3763
TEST (15%)		
n° samples	17	17
***r* (measured *vs.* predicted)**	0.5589	0.5594
SEP	0.7169	0.7537
RMSE	0.6420	0.6978
SB	0.12	0.12
NU	0.0003	0.0517
LC	0.4834	0.483

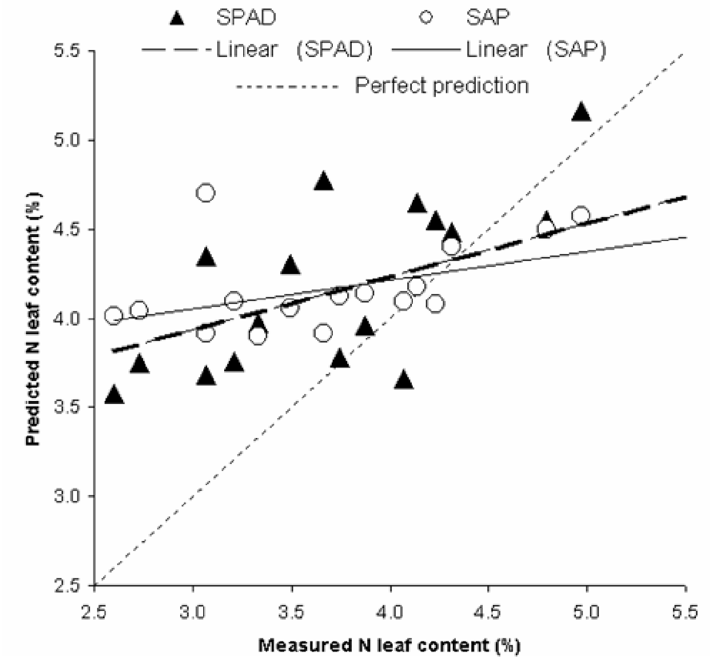

Figure 5.2.2.3.2: The correlation between measured and predicted values of N of SPAD (chlorophyll meter readings) and SAP (measurements of N-NO_3 concentration in petiole) analysis in the test represented by the 15% of the whole sample dataset extracted by the by the SPXY (sample set partitioning based on joint X- and Y-blocks) method.

Figure 5.2.2.3.2 shows the correlation between measured and predicted values of N by SPAD and SAP analysis in the test represented by the 15% of the whole sample dataset extracted by the SPXY method. In Table 5.2.2.3.2 values and results of PLS models and test prediction on the four spectral datasets (W, R1, R2 and R3) and of N concentration in tomato leaves from spectral reflectance analysis are reported.

Table 5.2.2.3.2: Results of Partial Least Squares (PLS) multivariate analysis on the four different datasets (W=whole dataset, 125 vars; R1=restricted dataset, first 92 vars, 400–694 nm; R2=restricted dataset, 46 vars, 400–694 nm, represented by the mean values of each single consecutive pair of steps; R3=restricted dataset, 62 vars, 496–694 nm) predicting the N concentration in tomato leaves from spectral reflectance analysis. In the table are reported: number of Latent Vectors (LV), Root mean square error in calibration (RMSEC) and validation (RMSECV), correlation coefficient (*r*), Standard Error of Prediction (SEP) and Root Mean Squares Error (RMSE).

	W	**R1**	**R2**	**R3**
n° total samples	117	117	117	117
n° LV	4	8	11	11
First pre-processing X-block	Log1/R	None	Log1/R	Log1/R
Second pre-processing X-block	sg	snv	sg	snv
Pre-processing Y-block	none	autoscale	autoscale	none
RMSEC	0.4942	0.5744	0.6079	0.4294
RMSECV	0.5120	0.7990	0.7924	0.5700
MODEL (85%)				
n° samples	100	100	100	100
***r* (measured *vs.* predicted)**	0.7436	0.8165	0.7917	0.8134
SEP	0.4967	0.4308	0.4533	0.4316
RMSE	0.4942	0.4286	0.4510	0.4294
TEST (15%)				
n° samples	17	17	17	17
***r* (measured *vs.* predicted)**	0.8856	0.8921	0.9244	0.9414
SEP	0.4186	0.4255	0.3597	0.3466
RMSE	0.4271	0.6460	0.5997	0.4054

The best model was the R3 using only the central values of the spectra (496–694 nm). This model uses firstly a Log1/r pre-processing on the X-block and then a SNV pre-processing on the pre-processed X-block. Y-block was not pre-processed. The *r* value of the test is very high: 0.94 (Table 5.2.2.3.2).

Also the values of SEP and RMSE of the test are very low (0.35 and 0.40 respectively). The prediction ability of the model revealed to be high, being a SEP of 0.43 and the RMSE of 0.43 indicates that predictions were on average within 0.43% N of the measured values. Moreover, the values of the predictive accuracy are equal to: SB=0.05, NU=0.0188 and LC=0.09.

5.2.2.4 Discussion and Conclusions

Usually N nutrition is determined by leaf chemical analysis, which presents some disadvantages that limit its use, such as the length of sampling time, the use of hand labour, the need for specialized equipment and high cost (Waskon et al., 1996). Thus, according to Guimarães et al. (1996) alternative methods that using portable gauges, permitting diagnosis and monitoring of the N nutrition of the plants in a faster and non-destructive way in the field are required.

In this study the estimation efficiency of the N concentration of tomato leaves determined by a portable VIS-NIR spectrophotometer by means of chemometric procedures resulted always higher than these obtained by SPAD chlorophyll meter readings and SAP tests, as demonstrated for the parameters r (0.94 *vs.* 0.56), SEP (near 40% lower), RMSE values (near 35% lower), SB (0.05 *vs.* 0.12), NU (0.0188 for VIS-NIR *vs.* 0.0003 for SPAD and 0.0517 for SAP test) and finally LC (0.09 for VIS-NIR *vs.* 0.48 for SPAD and SAP test). The N nutritional state in plants may be determined indirectly by the chlorophyll concentration present in the leaves, as it is directly related to their N concentration. Many studies found a high correlation between N and chlorophyll, because pigments determine most spectral features between 400 nm and 700 nm (Gausman, 1977; Yoder & Daley, 1989). It was confirmed in this work by proving that a restricted spectral dataset (R3=496–694 nm) that refers to the spectra range of the chlorophyll, highly correlated with the analysed leaf N concentration. Similar results were obtained in the study of Min et al. (2006) where the N leaf concentration of Chinese cabbage was detected using VIS and NIR spectroscopy in combination with PLS regression producing a r=0.92. The most significant wavelength correlated to chlorophyll was identified in the 710 nm, but also wavelengths near 550 and 840 nm contributed to N prediction as in our study.

Esposti et al. (2003) reported a SPAD chlorophyll meter for the multi-parametric chemical compound concentration estimation in leaves; they successfully estimated only N. However, the ability of this method to monitor crop N status in the field has been significantly enhanced by recent work analysing leaves of rice (Turner & Jund, 1991), corn (Wood et al., 1992a) and cotton (Wood et al., 1992b). The same situation is for the SAP test that have been developed to measure nutrient concentrations in a number of vegetable crops including potato (Williams & Meir, 1990), tomato (Lyons & Barnes, 1987), cabbage (Scaife & Stevens, 1983), cauliflower (Kubota et al., 1996) and capsicum (Olsen & Lyons, 1994). Both SPAD chlorophyll meter and SAP test are inexpensive and give rapid results which accuracy mainly depends on type, variety and phenological stage of the cultivation. Times of collection of petioles during the day for the SAP test are important if SAP nutrient concentrations show diurnal variation observed in beets (Minotti & Stankey, 1973) and also in tomato plants (Coltman, 1987). In addition, while plant N concentration declines with crop biomass accumulation, the N concentration per unit leaf area within the upper layer of canopy would remain more or less constant (Lemaire & Gastal, 1997); moreover, a vertical gradient in the canopy N concentration can be observed (Houlès et al., 2007). So the SAP test may be not able to show a decrease in plant N accumulation during the crop cycle since petioles are collected anytime at the top of the plants. Although many other factors can affect petiole nitrate concentration such as cultivar, temperature and solar radiation (Justes et al., 1997), the petiole SAP showed to be a reliable diagnostic tool for about 2/3 of the crop cycle (i.e., until the end of linear growth phase) when it is really important for N fertilizer management in processing tomato.

The tomato crop analyzed in this work resulted in a deficiency N concentration phase comparing with the critical N curve presented by Tei et al. (2002), especially until the early flowering period. Therefore, the estimation efficiency of the N concentration of tomato leaves determined by the SPAD and the SAP test, considering also the separate sampling period, was always underperforming respect to what indicated in literature. This fact could probably depend on the crop deficiency condition in terms of N concentration, referring to the critical N curve (Tei et al., 2002). The limited availability in the sample of elements with a concentration of leaf N exceeding critical thresholds may have limited the predictive power of both tests (SPAD and SAP).

In this study the utilization of the portable VIS-NIR spectrophotometer, increasing efficiency, allows better knowledge of nutritional status of tomato plants, with more detailed and sharp information and on wider areas. More detailed information either in space (increase in detail) and in time (the system allowed to perform spectral measurements with an acquisition time of 2 s per leaf, for 500–800 leaves and 100–150 plants) is an essential tool to increase and stabilize crop quality levels and to optimize the nutrient use efficiency, mainly in low input production models.

5.2.2.5 References

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6. Post-harvest

The post-harvest activities include all points, in the value chain, from production in the field to the food being placed on a plate for consumption (e.g., harvesting, handling, storage, treatments and underpinning mechanisms, processing, quality evaluation, packaging, transportation and marketing) (Mrema & Rolle, 2002).

Figure 6.1: Examples of post-harvest fruit processing machineries.

One among the major problems in the post-harvest chains is represented by the losses of horticultural produce because it is very difficult to measure. There are numerous factors affecting post-harvest losses, from the soil in which the crop is grown to the handling of produce when it reaches the shop. The reduction in post-harvest losses and the improvement of product quality became the main concern of producers, middlemen, marketing specialists and consumers.

Today, enormous volumes of quality horticultural crops produced in technologically advanced countries are made available to millions of people through improved post-harvest handling (Fig. 6.1). Thus, historically and by necessity, post-harvest technology is part of the normal development processes in agriculture. Appropriate production practices, careful harvesting and proper packaging, storage and transport all contribute to the good produce quality. Once a crop is harvested it is impossible to improve its quality. The horticultural crops, because of their high moisture content are inherently more liable to deteriorate. Moreover, they are biologically active and carry out transpiration, respiration, ripening and other biochemical activities, which deteriorate the quality of the produce. Losses during post-harvest operations due to improper storage and handling are enormous and can range from 5-35 percent. Post-harvest losses can occur in the field, in packing areas, in storage, during transportation and in the wholesale and retail market. Severe losses occur because of poor facilities, lack of know-how, poor management, market dysfunction or simply the carelessness of farmers. Proper storage conditions, temperature and humidity are needed to lengthen the storage life and maintain quality once the crop has been cooled to the optimum storage temperature (López Camelo, 2004).

It is distressing to note that so much time is being devoted to the culture of the plant, so much money spent on irrigation, fertilization and crop protection measures only to be wasted about a week after harvest. It is, therefore, important that post-harvest procedures be given as much attention as production practices the stages from planting until the product a roach the consuming public must be a mutual undertaking between the growers and those who will handle the products after harvest. The global demand for high fruit quality that are rich in nutrients and that can endure the demands of worldwide supply chains is growing rapidly (López Camelo, 2004).

6.1 Fruit quality evaluation

Quality could be defined as the “degree of fulfilment of a number of conditions that determine its acceptance by the consumer”. Here a subjective aspect is introduced because different consumers will judge the same product according to their personal preferences. The destination or use can also determine different criteria for judging quality within the same crop. It is common to use additional words to define the quality to the specific use such as industrial quality, nutritional quality, export quality and edible quality (López Camelo, 2004). Generally, quality is a complex perception of many attributes that are simultaneously evaluated by the consumer either objectively or subjectively (Figure 6.1.1). The brain processes the information received by sight, smell and touch and instantly compares or associates it with past experiences or with textures, aromas and flavours stored in its memory. For example, just by looking at the colour, the consumer knows that a fruit is unripe and that it does not have good flavour, texture or aroma. If colour is not enough to evaluate ripeness, the hands to judge firmness or other perceptible characteristics are used. The aroma is a less used parameter except in those cases where it is directly associated to ripeness. The final evaluation is the perception of the flavour, aroma and texture when the product is consumed and when sensations perceived at the moment of purchase are confirmed. Fruit and vegetables are consumed mainly for their nutritive value as well as for the variety of shapes, colours and flavours that make them attractive for food preparation. When they are consumed raw or with very little preparation, the consumer’s main concern is that they must be free of biotic or non-biotic contaminants that may affect health (López Camelo, 2004).

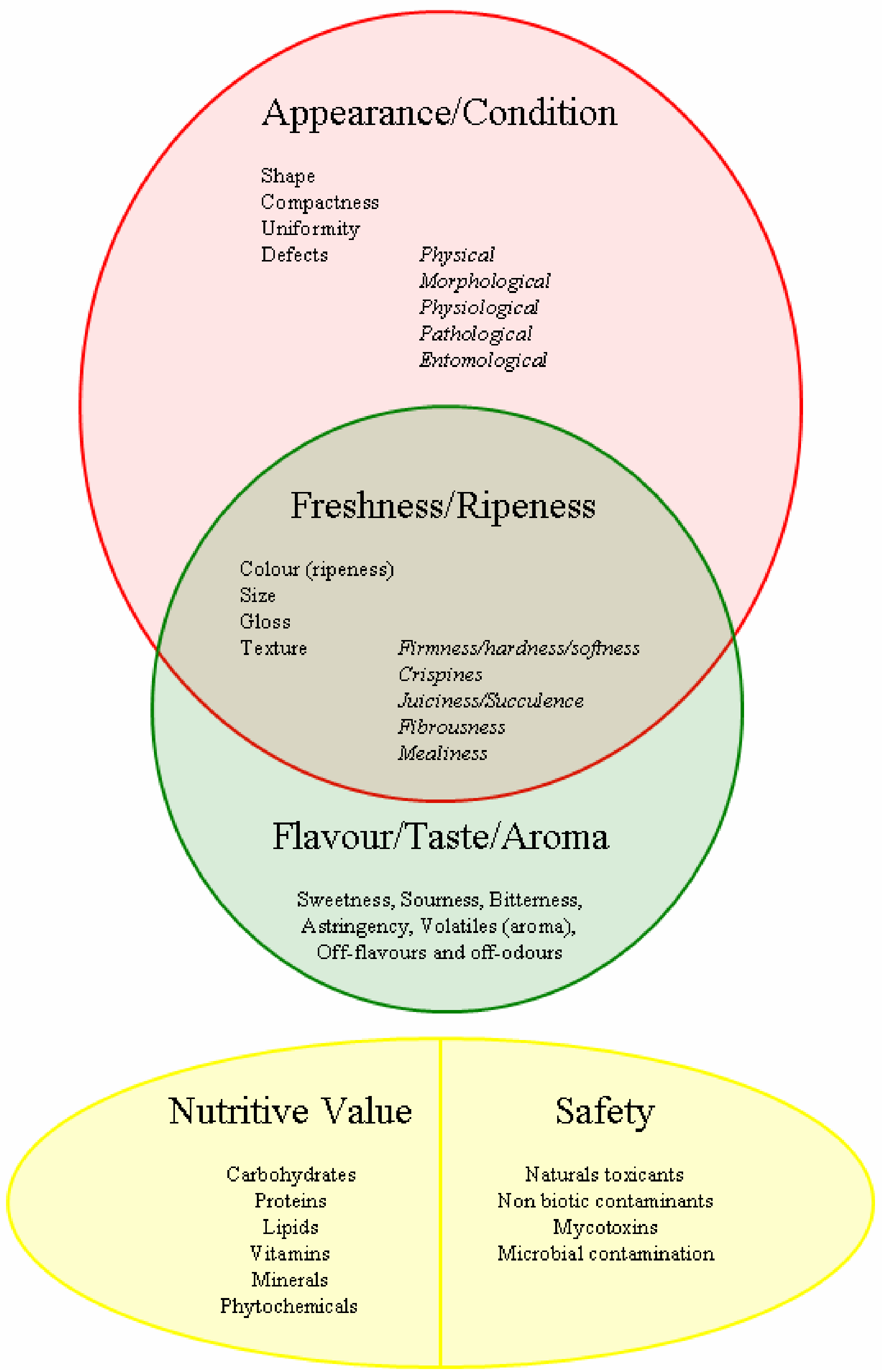

Figure 6.1.1: Consumer perception of quality (adapted from López Camelo, 2004)

The quality system established by the standards is known as "inspection for quality" where representative samples at the final stage of preparation for the market should fulfil the specified limits and their tolerances. Although it is easy to apply, this quality system has at least two major disadvantages. It is not totally adaptable for highly perishable products where quality varies continually, and its application does not improve the quality of the product, it only separates in degrees the quality that comes from the field (López Camelo, 2004). The conformity of agricultural products (i.e. in terms of morphological-organoleptic homogeneity) is represented by the sum of several biological parameters that must satisfy a quality standard criterion in order to be considered acceptable by consumer. The conformity sets a level of restriction, since it implies a certain number and type of valuable parameters, which depend upon the commercial context and its legislation. Once the international law is satisfied, each state, region or large organized distribution chain can apply a more restrictive version of it. Indeed, the chains of trading enterprises that count on several average or big centres deal with the large-scale distribution and need to provide products with constant characteristics during time, namely, with a defined conformity in relation to a reference standard. Condition is a central aspect of product conformity, but its definition is still subjective (Costa et al., 2011). Generally the most important components of fruit quality are the appearance and the nutritive value (Fig. 6.1.1).

As reported by Kays (1991) the appearance of fresh fruit and vegetables is a primary criterion in making purchasing decisions. Product appearance is characterized by size, shape, form, colour, condition and absence of defects. Appearance is utilized throughout the production storage marketing utilization chain as the primary means of judging the quality of individual units of product. In this context, the appearance of unities of products is evaluated by considering their size, shape, form, colour, freshness condition and finally the absence of visual defects. All these characteristics contribute to the overall appearance, which is globally evaluated either in a metric or a subjective manner as an important quality indicator (Costa et al., 2011).

The shape of agricultural products is one of the most important factors for their classification and grading in relation to commercial quality and organoleptic properties (Morimoto et al., 2000). Irregularities in shape are a critical factor in consumer decision. Less pronounced shape defects are not perceived, while on the contrary, more extreme variations may deeply influence purchasing decision, leading to the ultimate rejection of a product (Kays, 1999). Compactness it is not associated to their organoleptic characteristics but rather is an indicator of the degree of development at harvest. To a certain extent, compactness is also an indicator of freshness because it decreases with dehydration. Uniformity is a concept applied to all the components of quality (size, form, colour, ripeness, compactness, etc.). For the consumer it is a relevant feature that indicates that someone who knows the product has already selected and separated it into categories based on the official standards of quality. It is so important that selecting products for uniformity is the main activity of market preparation. Internal or external defects do not affect product excellence, but the consumer rejects them because the absence of defects is one of the main components of appearance and therefore of the primary decision to purchase. Different factors during growth (climate, irrigation, soil, variety, fertilization, etc.) can lead to morphological or physiological defects (López Camelo, 2004).

Generally, the appearance is the most simple and fast parameter to be monitored but not the only in order to obtain a quality product. In fact, another important parameter to determine fruit quality in post-harvest operations is represented by the analysis of the nutritive value. Generally, from the point of view of nutrition, fruit and vegetables are insufficient to satisfy daily requirements, essentially because of their low content of dry matter. They have a high content of water and are low in carbohydrates (except for sweet potatoes, potatoes, cassava, and other underground organs), proteins (except for legumes and some crucifers) and lipids (except avocados), but, in general, they are a good source of minerals and vitamins. The content of soluble solids is a good estimate of total sugar content, and many fruit should have a minimum content of solids to be harvested. Organic acids (citric, malic, oxalic, tartaric) are the other important components of taste, particularly in their relationship with soluble solids. As the fruit ripens the organic acids tend to diminish and so the relationship with the soluble solids tends to increase. Titratable acidity is the form of expressing acidity. The soluble solids/titratable acidity relationship is a denominated ratio and it is essentially used in citrus where it is a function of the species and of the variety (Lacey et al., 2000).

6.1.1 Why evaluate fruit quality?

Producing a quality product begins well before planting the seed. Soil selection and preparation, its fertility and irrigation potential, weed control and crop rotations, variety selection and other decisions have an influence on the quality of the product. In the same way, quality is affected by the climatic conditions during the growing period, as well as irrigation, fertilization, control of pests and diseases and other cultural practices. Harvest is the end of cultivation and the beginning of post-harvest actions during which preparation for the market, distribution and sale take place. Fruit are highly perishable products that demand water and nutrients before being detached from the mother plant. Once harvested, however, they depend on their reserves to continue living. Post-harvest changes can only be delayed within certain limits and thus preparation for the fresh market should be quick and efficiently performed in order to avoid especially the internal quality losses (López Camelo, 2004). The improvement of non-destructive, rapid and cost-effective analysis for the determination of fruit internal quality (represented for example by the total soluble solids content and the titratable acidity) could represents an important advance in fruit marketing, allowing fruit quality classifying not only on the base of appearance, but also in relation to gustative characteristics. This makes farmers aware of the possibility to gain a premium price for the fruit internal quality and not only for their aesthetical attributes, the simplest parameters to detect in the monitoring of product quality.

6.2 Case study

For the post-harvest phase one case study was developed in order to assess possibility and limits of a non-destructive estimation of citrus fruit internal quality parameters (i.e., total soluble solids and titratable acidity) presenting thick peel by the use of a spectrophotometric portable VIS-NIR system (Fig. 1.1).

6.2.1 Antonucci F, Pallottino F, Paglia G, Palma A, D'Aquino S & Menesatti P (2011) Non-destructive estimation of mandarin maturity status through portable VIS-NIR spectrophotometer. Food and Bioprocess Technology, 4(5), 809-813. (IF2010=3,576)

Abstract

Sugar content is one of the most important quality attributes of citrus fruit, either for fresh or for processing market. Since sugars in citrus juice are highly correlated with total soluble solids (TSS) content, which can be determined easily even by the means of a hand refractometer, TSS is one of the most frequently used quality index. Since TSS can be measured only destructively, the results are representative only if carried out on large samples and do not allow classifying marketable fruit one by one according to their specific sugar content. Objective of this experiment was to assess possibility and limits of a non-destructive estimation of citrus fruits internal quality parameters (TSS and titratable acidity) presenting thick peel by the use of a spectrophotometric portable VIS-NIR system. Four hundred fruit of "Miho" satsuma and 150 fruit of "Page" tangelo were used. Each fruit was first subjected to spectrophotometric acquisition and soon after was juiced and TSS and titratable acidity (TA) determined. Partial least squares (PLS) regression analysis was applied for constructing a predictive model based on the spectral normalized response, constructing the model on a sub-sample and verifying the model (prediction test) on independent ones. The TA relative to Page mandarin was predicted in the test with an r=0.88 and a standard error of prevision (SEP) coefficient of variability of 3.8% while the TSS scored an r=0.85 and a SEP coefficient of variability equal to 4%. The TA of Miho mandarin was predicted in the test with an r=0.81 and a SEP coefficient of Variability of 8.3% while the TSS scored an r=0.84 and a SEP coefficient of variability equal to 5.6%.

Keywords: Mandarin, VIS-NIR, Partial least squares, Total soluble solids, Titratable acidity.

6.2.1.1 Introduction

Besides the appearance conferred to the fruit by size, color, shape and surface defects, total soluble solids (TSS), directly related with the sugar content, and titratable acidity (TA) are crucial attributes indicating the fruit internal quality (Nicolaï et al., 2007). Non-destructive optical methods based on visible/near-infrared spectroscopy (VIS/NIRS) have been evaluated for non-destructive estimation of internal starch, soluble solids content, oil contents, water content, dry-matter content, acidity, firmness, stiffness factor, and other physiological properties of a number of fruit and vegetable products indistinctly including citrus (Steuer et al., 2001; Miller & Zude-Sasse, 2004; Cayuela, 2008; Lu et al., 2008; Zude et al., 2008) mandarin (Kawano et al. 1993; McGlone et al. 2003); tomato (Slaughter et al. 1996); mango (Saranwong et al., 2004); kiwifruit (Osborne & Künnemeyer, 1999); apple (Lammertyn et al., 1998; Park et al., 2003; Menesatti et al., 2009).

In citrus, fruit juice content, TSS and TA are the main internal quality parameters used all over the world. Packinghouse managers take representative samples of fruit to test internal quality before shipping, but controversy arises when the required minimal level of TSS is not met or when TA exceeds the highest tolerated level. Unfortunately for the same cultivars TSS and TA vary greatly in fruits harvested from the same tree and this variability depends on a large number of factors such as the length of the blooming period, which causes the setting of fruits of different age in the same tree not easily discernable at the picking time, the position of the fruits in the canopy, on the kind of inflorescences which bear the fruit. A reliable nondestructive analysis means would allow to select fruits according to their real and individual quality characteristics and to match the required internal quality standards.

The aim of this study was to evaluate the ability of VIS/ NIR spectroscopy to determine TSS and TA of two thick peel mandarin cultivars. The results, elaborated through partial least squares (PLS) analysis, have been compared with standard destructive techniques to assess the respective quality characteristics and to obtain the prediction models.

6.2.1.2 Materials and Methods

The fruit were harvested on 27th November 2003 from trees grafted onto sour oranges cultivated at the CNR’s experimental station (Oristano, west Sardinia). After harvest, fruit were immediately delivered at the laboratory where 400 of “Miho” satsuma and 150 of “Page” tangelo free of visible defects were chosen for the experiment. For the VIS/NIR measurements, a portable single channel spectrometer was used (Fig. 6.2.1.2.1). It performs point measurements of different emitted light quotes, in function of the probe applied: reflectance and standard color CIELAB 45/ 0, absorbance and interactance. The system is composed of five parts: (1) a spectrograph Hamamatsu S 3904 256Q in a special housing; a customized illumination system realized by a 20 W halogen lamp and an optical fiber bundle consisting of approximately 30 quartz glass; (2) an optical entrance with input round: 70×2,500 μm and diameter 0.5 mm NA=0.22 mounted in SMA-coupling; (3) specific probes with quartz optical fiber of connection; (4) a transmission device for transmitted or absorbed light for thin solids or liquid with variable optical length; (5) a notebook equipped with specific software to acquire, calibrate, and elaborate spectral data. The Hamamatsu spectrograph has the following characteristics: grating: Flat-field, 366 l/mm (center); spectral range: 310–1,100 nm; wavelength accuracy absolute: 0.3 nm; Temperature induced drift: < 0.02 nm/K; Resolution (Rayleigh criterion): DlRayleigh > 10 nm; sensitivity: > 1013 Counts/ Ws (with 14-Bit-conversion); straylight: < 0.8% with Halogen lamp and A/D converter 16 bit. For spectral acquisition, the 'pen' probe was used to measure the spectral reflectance response on each single fruit (spot area≈10 mm2): twice in different equatorial parts. The diffuse reflectance measure is referred to the light diffuse quote that is reflected by the material and acquired by an optical quartz fibre (0.7 mm in diameter) fixed at 45° inside a circular aperture of 4 mm in diameter. The material surface due to its softness was able to include all the circular aperture avoiding any external light interference. Measurements were performed placing the probe's head perpendicularly to the fruit surface to avoid external light noise. These spectral measurements were performed in laboratory considering a white calibration (small variable in function of the external light), the instrumental integration time (light acquisition time), and subtracting the background noise (variable in function of the instrument temperature) (Fig. 6.2.1.2.1). A very low signal/noise ratio was observed in the beginning and at the end of the spectral data, affecting the accuracy measurements, so only the spectrum in the range 400-1,000 nm were take into account for the analysis.

Figure 6.2.1.2.1: **A)** Portable VIS-NIR spectrophotometer. **B)** Trained operator performing measurements on mandarin fruit. **C)** Pen probe head acquiring the signal. **D)** Fibre light emitter.

All spectral values were expressed in terms of relative reflectance (R) (Menesatti et al. 2009). Reflectance measurements were compared to standard chemical value averaged on single fruit. All fruits were individually numbered and after taking all the measurements (readings) for non-destructive analysis were juiced and chemical analysis were performed on the juice of each single fruit in order to verify if correlations existed between non-destructive parameters and the real values of TA and TSS determined destructively. Estimation of chemical levels was performed by PLS regression model on the basis of the reflectance spectral values (Pallottino et al., 2010).The dataset was randomly separated into two subsets, one used for the model, 75% of the whole dataset, and the remaining 25% used for the independent validation test. The sample data was separated randomly into two groups: a calibration set used to develop the calibration models and the remaining samples of the population were used as prediction sets. The calibration models were also validated using full cross-validation. The x- and y-blocks were generally pre-treated using Autoscale, that centres columns to zero mean and scales to unit variance. In the case of the Page xblock was used a Savitzky-Golay smoothing and differentiation which performs a smoothing on the matrix of row vectors y. At each increment a polynomial of order is fitted to the number of points width surrounding the increment.

6.2.1.3 Results and Discussion

Table 6.2.1.3.1 shows the results and the parameters used to build the PLS model relatively to the TA and TSS estimation for the Page mandarins analyzed.

Table 6.2.1.3.1: Results of PLS prediction relative to the titrable acidity (TA) and total soluble solids (TSS) of the PAGE samples.

PAGE				
	TA		TSS	
PLS Model parameters				
	MODEL	TEST	MODEL	TEST
n° of samples	99	24	99	24
X-block variables	185		185	
latent variables	15		8	
Pre-processing X-block	Savitzky-Golay		Savitzky-Golay	
Pre-processing Y-block	Autoscale		Autoscale	
X-block % cum. var. captured	100.00		99.97	
Y-block % cum. var. captured	90.96		68.46	
RMSEC	0.02		0.38	
RMSECV	0.09		0.45	
***r* (predicted vs. measured)**	0.95	0.88	0.83	0.85
SEP Coefficient of Variability (%)	2.5	3.8	3.7	4
RMSE	0.02	0.03	0.38	0.40

The correlation coefficients (*r*) between observed and predicted values were equal to 0.88 for the TA (Fig. 6.2.1.3.1a) prediction and 0.85 for the TSS (Fig. 6.2.1.3.1b) for the independent test.

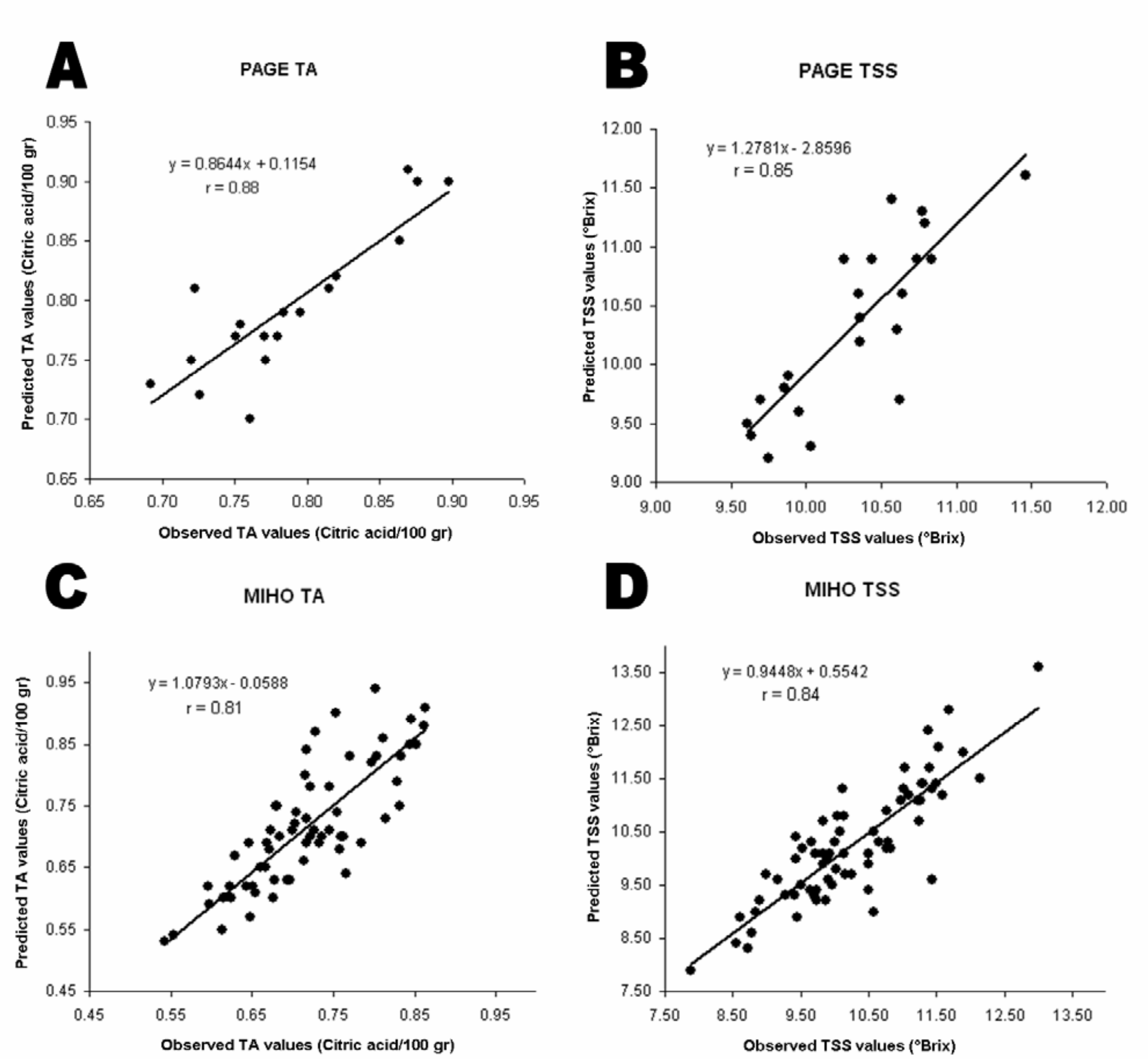

Figure 6.2.1.3.1: Linear regressions between the predicted and the observed values of the: A) TA, Page; B) TSS, Page; C) TA, Miho; D) TSS, Miho.

The number of the LV used to build the model was lower for the TSS (8) then for the TA (15). The SEP coefficient of variability, calculated as the SEP divided by the average of the total observed values, was found for the Page test to be lower in the TA case in comparison with the TSS and respectively of 3.8% and 4%. Table 6.2.1.3.2 reporting the results relative to the Miho estimation shows a number of latent variables equal in both TA and TSS model prediction (15).

Table 6.2.1.3.2: Results of PLS prediction relative to the titrable acidity (TA) and total soluble solids (TSS) of the MIHO samples.

MIHO				
	TA		TSS	
PLS Model parameters				
	MODEL	TEST	MODEL	TEST
n° of samples	320	80	320	80
X-block variables	185		185	
latent variables	15		15	
Pre-processing X-block	Autoscale		Autoscale	
Pre-processing Y-block	Autoscale		Autoscale	
X-block % cum. var. captured	99.99		99.99	
Y-block % cum. var. captured	45.16		87.39	
RMSEC	0.04		0.36	
RMSECV	0.07		0.59	
***r* (predicted vs. measured)**	0.89	0.81	0.93	0.84
SEP Coefficient of Variability (%)	5.6	8.3	3.5	5.6
RMSE	0.04	0.06	0.36	0.58

The correlation coefficient (*r*) in the independent test between observed and predicted values was 0.81 for TA (Fig. 6.2.1.3.1c) prediction and 0.84 for TSS (Fig. 6.2.1.3.1d). In this case, the SEP coefficient on variability for the test resulted lower for TSS, 5.6%, with respect to TA, 8.3%. Therefore, the highest correlation coefficient (*r*) between observed and predicted values was identified for the TA prediction of Page.

Internal quality features such as total soluble solids content, sugar content, juice acidity, dry-matter content and firmness are well known to be crucial attributes of the fruit (Gómez et al., 2004). The results of this study showed that a system based on a portable spectrophotometer can provide better knowledge of mandarin quality, achieving a more detailed and focused quanti-qualitative information in a shorter period of time. The autonomy of the instrument, taking into account the time needed to move from one fruit to the other, allows date acquisitions to perform on about 1,200-1,300 fruit. The power supplied by the portable batteries of the instrument and the notebook computer, guaranteed a working period of about 1.5 h. 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, obtaining a highly representative sample. 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 select fruit on the base of their quality fastening an important post-harvest operation.

Most instrumental techniques to measure such properties are destructive thus, involving a huge amount of manual work. In this study, a non-destructive method based on a high number of VIS/NIRS has been evaluated to estimate two of these important internal quality features: soluble solids content and acidity.

6.2.1.4 Conclusions

The improvement of non-destructive analysis for the determination of TSS and TA do represents an important advance in market of citrus fruit, allowing fruit quality classifying not only on the base of visual aspect, but also in relation to gustative characteristics. This could lead to changes in agricultural methods and strategies, making farmers aware of the possibility of gaining a premium price for the internal quality of the fruit and not only for their aesthetical attributes.

6.2.1.5 References

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7. Overall conclusions and recommendations

Agriculture plays a crucial role in maintaining the environment healthy having contributed over the centuries to create a variety of valuable semi-natural habitats. For these reasons, in the last years, the demand of new technologies, characterised by innovative analytical and quality control strategies, strongly increased in order to permit to the agro-environmental systems to remain sustainable and productive in a long term period.

In this Ph.D. research the utilization of a branch of these new methodologies, in particular of the optoelectronic techniques, increasing efficiency and decreasing environmental impact, allowed better knowledge in all the agricultural chain "*from the cradle to the grave*" in particular in three central phases: in-field soil preparation, pre-harvest and post-harvest activities. Three of the most important agro-environmental production parameters were monitored: soil water content, crop nutritional status and fruit quality. This was done through optoelectronics techniques allowing a faster and finer acquisition of data with respect to conventional ones on wider areas.

The results published in the international peer-reviewed journal *Sensors* for the in-field soil preparation have demonstrated as both thermography and thermometry are innovative techniques that could produce highly informative results about soil water content if paired with a Geographic Information System (GIS). Soil water content is an important factor used in agriculture to make decisions on crop planting, irrigation scheduling and soil stability for agricultural machinery operations. In addition, the GPS receivers, used to locate and navigate agricultural vehicles in-field, have become commonly used sensors in precision agriculture. When a GPS receiver and a data logger are used to record the position of each soil sample or measurement, a map can be generated and processed along with other layers of spatially variable information (map-based approach). On the other hand, some soil sensors may be used to vary application rates in response to sensor output in real time without a GPS receiver (on-the-go soil real-time approach).

The next step in the agricultural chain is represented by the pre-harvest activities. Generally, pre-harvest production practices may seriously affect post-harvest returns in particular for the fruit quality parameter. For this reason, a good monitoring of crop nutritional status during pre-harvest activities could lead to an excellent final product to harvest. The two studies conducted and published on two different international peer-reviewed journals (i.e., *Biosystem Engineering* and *Sensors*) represent a good application for crop nutritional status monitoring during the pre-harvest phase, through a rapid, non-destructive and cost-effective technique. Such studies performed a foliar analysis on Tarocco orange trees and processing tomatoes using a portable VIS-NIR spectrophotometer coupled with multivariate statistical analysis. The results obtained with these advanced technologies were compared with those obtained by standard chemical analyses. From such comparison appear as the innovative methods used although score similar results to those of the standard ones were very satisfactory being faster, less expensive and non-invasive. Especially during pre-harvest operations the time response of an analysis pointing to evidence the plant nutritional status is crucial in order to allow a correct fertilization scheduling in the shortest time possible.

Finally the methodologies presented in this Ph.D. work dealt with fruit quality monitoring during post-harvest operations. The results of this part of the research were published on the international peer-reviewed journal *Food and Bioprocess Technology*. The improvement of non-destructive, rapid and cost-effective analysis for the determination of fruit internal quality (e.g., the total soluble solids content and the titratable acidity) represented an important advance in fruit marketing, allowing fruit quality classifying not only on the base of their appearance, but also in relation to gustative characteristics. This makes farmers aware of the possibility to gain a premium price for the fruit internal quality and not only for their aesthetical attributes, the simplest parameters to detect in the monitoring of product quality.

Concluding these three years of Ph.D. activities has provided the basis for future applications on:

- monitoring and stabilising the crop nutritional levels (appropriate fertilisation strategy);
- assessing rapidly the soil water content (appropriate irrigation scheduling);
- assessing the fruit internal quality (higher quality fruit stock with increased value);
- reducing the impact on the agro-environmental systems (avoiding the over ferti-irrigations).

These strategies, being already good agricultural practices, find their natural application especially if used on site-specific management tasks, as required in precision farming to obtain detailed information about the heterogeneity of crop and soil and drastically reduce the use of chemicals.

The proposed methodologies resulted very interesting for the limited time of execution, needed to obtain results on dynamic crop and soil characteristics, making these implementable on commercial machine systems for very expeditious in-field applications. Their use along the three step of the production agricultural chain investigated results in a synergic effect.

8. List of publications

8.1 International peer-reviewed scientific publications with Impact Factor (IF)

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15. Costa C, **Antonucci F**, Menesatti P, Pallottino F, Boglione C & Cataudella S (In press) An advanced colour calibration method for fish freshness assessment: a comparison between standard and passive refrigeration modalities. In press on Food and Bioprocess Technology. DOI 10.1007/s11947-011-0773-6. (IF2010=3,576)
16. Pallottino F, Costa C, **Antonucci F**, Strano MC, Calandra M, Solaini S & Menesatti P (In press) Electronic nose application for determination of *Penicillium digitatum* in Valencia oranges. In press on Journal of the Science of Food and Agriculture, DOI 10.1002/jsfa.5586. (IF2010=1,360)
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8.2 Peer-reviewed scientific publications

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2. Marino G, Boglione C, Livi S, Palamara E, De Innocentis S, Costa C, **Antonucci F**, Di Marco P, Petochi T & Cataudella S (2011) Vallicoltura: an endangered aquaculture practice? Aquaculture Europe, 36(1), 19-23.
3. Milanesi C, **Antonucci F**, Menesatti P, Costa C, Faleri C & Cresti M (2012) Morphology and molecular analysis of ancient grape seeds. Interdisciplinaria Archaeologica, Natural Sciences in Archaeology, 2(2): 145-150.

8.3 Others scientific publications

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2. Costa C, Pallottino F, Angelini C, Proietti M, Capoccioni F, Aguzzi J, **Antonucci F** & Menesatti P (2009) Colour calibration for quantitative biological analysis: a novel automated multivariate approach. Instrumentation Viewpoint, 8: 70-71.
3. **Antonucci F**, Pallottino F, Canali E, Giorgi S & Menesatti P (2009) RFID, fresh cut più efficiente. Colture Protette, orticoltura e florovivaismo. Edagricole. Mensile de “Il Sole 24 Ore Business Media” anno XXXVIII/ottobre 2009, pp 58-61.
4. Pallottino F, **Antonucci F**, Canali E, Giorgi S & Menesatti P (2009) PRS per prodotti deperibili. Colture Protette, orticoltura e florovivaismo. Edagricole. Mensile de “Il Sole 24 Ore Business Media” anno XXXVIII/ottobre 2009, pp 50-57.
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8.4 Congress oral communications and Posters

1. Aguzzi J, Costa C, Company JB, **Antonucci F**, Pallottino F, Menesatti P, Canali E, Giorgi S, Angelini C & Ketmaier V (2009) Application of geometric-morphometric, hyperspectral imaging and molecular markers to the study of depth-driven differences in populations of Decapods (Crustacea). Poster at III International Workshop on Marine Technology, MARTECH09 19-20 November 2009, Vilanova i la Geltrù (Barcelona), Spain.
2. Menesatti P, **Antonucci F**, Costa C, Santori A, Niciarelli I & Infantino A (2009) Application of morphometric image analysis system to evaluate the incidence of fusarium head blight wheat infected kernels. Poster at 1st International Workshop on Computer Image Analysis in Agriculture, Potsdam, Germany 27-28 August 2009, Bornimer Agrartechnische Berichte-Heft 69, ISSN 0947-7314, Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V. (ATB).
3. Costa C, **Antonucci F**, Pallottino F, Canali E, Boglione C, Cataudella S & Menesatti P (2010) An innovative colorimetric calibration method used to quantify differences among wild and reared sea breams. Poster at XVII World Congress of the International Commission of Agricultural Engineering (CIGR), June 13-17 2010, Québec City, Canada. Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB).

4. Ulissi V, Benincasa P, Guiducci M, **Antonucci F** & Menesatti P (2010) Estimation of tomato nutritional status by VIS-NIR portable spectrophotometric system. Oral presentation at XVII World Congress of the International Commission of Agricultural Engineering (CIGR), June 13-17 2010, Québec City, Canada. Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB).
5. **Antonucci F**, Menesatti P, Canali E, Giorni S, Maienza A & Stazi SR (2010) Hyperspectral imaging characterization of agricultural topsoil copper concentration. Poster at 2nd International Workshop of the International Commission of Agricultural Engineering (CIGR), 26-28 August 2010, Budapest, Hungary.
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9. **Antonucci F**, Pallottino F, Costa C, Vincenti F, Iacurto M, Canali E & Menesatti P (2011) A new RGB calibration for the rapid and low-cost color imaging of beef meat. Oral presentation at 6th International CIGR Technical Symposium, Section 6, Towards a Sustainable Food Chain, April 18-20 2011. Food Process, Bioprocessing and Food Quality Management Nantes, France.
10. Pallottino F, Moresi M, Lanza MC, **Antonucci F** & Menesatti P (2010) Classificazione Reometrica di Arance Rosse: Analisi Strumentale e Sensoriale a Confronto. Poster at III Convegno Nazionale di Scienze Sensoriali, 1-2 December 2010. Università degli Studi di Napoli Federico II, p 37. (Award for best presented work).

11. Pallottino F, **Antonucci F**, Costa C, Giorgi S & Menesatti P (2011) Colorimetric analysis of sweet cherry stem darkening: a tool for freshness evaluation. Proceedings of the 7th National Color Conference. Gruppo del Colore, SIOF, www.gruppodelcolore.it, Sapienza Università di Roma, Facoltà di Ingegneria, Roma, Italy, 15-16 September 2011. Colour and Colorimetry. Multidisciplinary Contributions, edited by Maurizio Rossi, Dip. Indaco, Politecnico di Milano, 7B, 343-348.
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14. **Antonucci F**, Marabottini R, Menesatti P, Petruccioli M, Giorgi S & Stazi SR (2012) Hyperspectral imaging characterization of agricultural topsoil arsenic concentration with *ad hoc* contaminated soils. Poster at EUROSOIL 2012, 4th International congress of the European Confederation of soil science Societies (ECSSS), 2-6 July 2012, Bari, Italy.
15. Menesatti P, Giorgi S, Burchi G, Prisa G, Canali E, Pallottino F, **Antonucci F** & Costa C (2012) An innovative application of passive refrigeration system to preserve fresh-cut flowers. Poster at the International Conference of Agricultural Engineering CIGR-AgEng2012, July 8-12 2012, Valencia, Spain.
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8.5 Books

1. Menesatti P, Pallottino F, **Antonucci F**, Roccuzzo G, Intrigliolo F & Costa C (2012) Non-destructive proximal sensing for early detection of citrus nutrient and hydric status. In: Srivastava AK (ed), Advances in Citrus Nutrition. Springer-Verlag, The Netherlands.

8.6 Patents

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2. Costa C, Menesatti P & **Antonucci F** (30 Dicembre 2011) Deposito della domanda di brevetto per Modello di Utilità dal titolo: "Dispositivo di Protezione Individuale - Stivali impermeabili ad apertura rapida". Domanda n°RM2011U000205.

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