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Two Case Studies of Precision Horticulture Approaches in Citrus Production

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ABSTRACT

In the age of information technology, process-oriented data analysis is the focus of emerging science and is predicted to form the basis for economic growth. The general consensus is that, especially in agronomy, new innovative approaches are needed for a future process management. Because citrus is economically a very important fruit crop worldwide, two case studies of *in-situ* analysis of plant and fruit with prominent importance in the citrus production are reviewed targeting site-specific production measures. In the first approach, the review provides a background of the measurable plant response to oxygen shortage in the root zone by means of non-destructive methods and gives an outlook of the applications in citrus. In a second case study the quality analysis of citrus fruit at the tree level is discussed. The feasibility of the spatio-temporally resolved data recording in the harvest management is pointed out.

Keywords: citrus fruit quality, non-destructive, oxygen shortage, spatio-temporal, site-specific, soluble solids content

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INTRODUCTION

In comparison to other industry sectors, technological advances in agricultural and horticultural production appear very gradually. A reasonable concept has been introduced in the framework of precision agriculture. Here, the plant itself is monitored *in-situ* to adapt the production measures to the needs of the plant. Biological variability and environmental impacts are taken into account for the first time by means of data collected spatially and temporally resolved, leading to more sustainable production processes (Stafford 2000; Grenzdörffer and Gebbers 2001; Zhang *et al*. 2002; Ewert *et al*. 2005; Rounsevell *et al*. 2006).

Such change in the point of view would be necessary also in the horticultural production. Approaches are presently developed taking into account a slightly increasing number of publications (Cook and Bramley 1998; Hall *et al.* 2002; Hense *et al*. 2002; Lamb *et al*. 2004; Herold *et al.* 2005; Bramley and Hamilton 2007; Reynolds *et al.* 2007; Gebbers and Zude 2008; Qing *et al.* 2008; Zude *et al.* 2008a). In horticulture, high investment costs, high productivity of the land area and highly perishable, valuable, and heterogenous produce provide even better reasons for high-technology production methods. The high heterogeneity of the horticultural produce often requires even batch-wise handling (Tijskens and van Kooten 2006; Schouten *et al.* 2007). The product quality during production is determined by plant genome, environmental conditions, and production system leading to high variability at harvest time (**Fig. 1**). Plant analyses in the laboratory are already important components of the process management in, e.g., plant nutrition and postharvest handling of produce. However, this kind of random spots measuring does not comprise an up-to-date solution for sufficient controls with temporal high-resolution analysis.

Nowadays, the discipline of non-destructive plant and product monitoring under various conditions is developing rapidly. Depending on the observation level, spectral-optical methods have been applied in remote sensing (e.g. Omasa *et al.* 2007; Verstraeten *et al.* 2008) for canopy screening as well as in molecular analysis of a diagnostic plant parameter under question (e.g. Krause and Weis 1991; Lichtenthaler and Schweiger 1998; Abbott 1999; Saito 2006; Lenk *et al.* 2007; Zude 2009).

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Invited Review

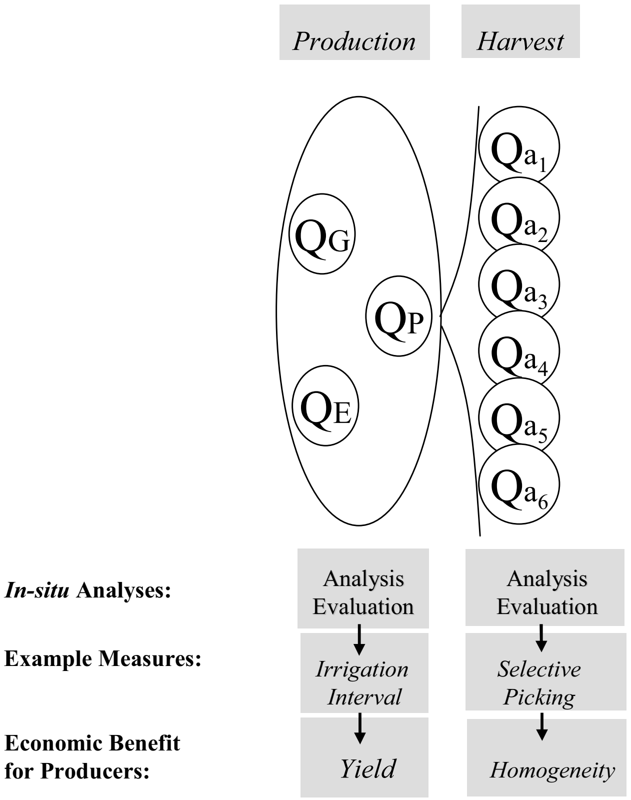

Fig. 1 Quality of perishable products with major factors in the production. $Q_{E,\ G,\ P}$ = Factors influencing quality (environment, genome, production measures), Qa = apparent product quality at harvest time (Zude and McRoberts 2006).

Machine vision systems have been established for sorting as well as monitoring during crop production and processing. Research groups in cooperation with industry have recently developed new sorting lines using spectroscopic methods, e.g., for grading according to fruit soluble solids (Birth and Hecht 1987; Ozaki *et al.* 2006; Nicolai *et al.* 2007) and pigment contents (Cubeddu *et al.* 1999; Merzlyak *et al.* 1999; Eccher Zerbini *et al.* 2002; Zude 2003; Zude *et al.* 2007; *c.f.* Torricelli 2009). Desktop modules and portable instruments for individual product testing became available in the past five years based on the same technology. As a result, internal quality parameters of fruits can be assessed directly in the production process and subsequently checked in the entire supply chain. It is precisely this repeated analysis along the supply chain that is essential to assess the impact of process conditions in production and postharvest on the product quality. Furthermore, new innovative optical compounds (Sumpf 2009), recent generic modeling attempts regarding the interactance of light and matter (Fukhanski *et al.* 1993; Qin and Lu 2007a, 2007b; *c.f.* Torricelli 2009), and logistic data processing methods for spatio-temporal resolved analyses (e.g. Piepho *et al.* 2003; Minasny *et al.* 2005; Trauth *et al.* 2007) provide the means to reach the next level of fruit production.

With increasing demands regarding the land use and water resources as well as high crop quality requests, the production methods are changing. Traditional citrus farming with trees planted in square with, e.g., 25 m × 25 m space has been replaced by systems with higher tree density supported by water-efficient drip irrigation. Intensive production systems using adapted cultivars and pruning techniques show densities up to 1,300 trees per acre, which is in the range that we find in apple production with small spindle tree forms. The advantage of intensive production system is to grow fruit in a more economic way as well as to be able to manipulate the growing conditions for optimizing the fruit quality and yield. The drawback is the increasing susceptibility of the system against unfavorable conditions, since the soil capacity to work as a buffer is diminishing. Taking into account the enhanced investment costs, the introduction of sensors for *in situ* analysis appears more and more reasonable.

The following sections will point out two applications for *in-situ* analyses of plant responses, approaching the non-destructive diagnosis of the physiological stage of plant and produce.

PRECISION HORTICULTURE FOR CITRUS: COPING WITH OXYGEN LIMITATIONS IN THE ROOT ZONE

Water logging in irrigated horticulture

Water supply is one of the major impacts on plant growth under field conditions. Drought stress was recently reviewed (Davies *et al.* 2005; Mahajan and Tuteja 2005; Parry *et al.* 2005; Salekdeh and Komatsu 2007; Neumann 2008). On the other hand, a serious horticultural problem is that of temporary flooding. The lack of oxygen (anoxia) in the root zone linked to submerged soil can lead to considerable yield losses. Citrus, in particular, is susceptible to oxygen deficiency in the root environment. Symptoms of different stress factors are often similar. To distinguish between an excess and deficit of water is difficult, but naturally is even more difficult by means of soil, instead of plant data.

Drip irrigation in hydroponic systems and intensive field production, flooding events and high ground water level in the rainy season, lead to limited soil gases exchange rates. Under these conditions, equilibrium of oxygen partial pressure from atmosphere to rhizosphere is rather a result of O_2 diffusion in soil solution than convection, because oxygen transport through air filled pores is impeded. Oxygen diffusion in solution is 11,300 times reduced in comparison to movement in air. As a result, flooding causes a reduced soil oxygen partial pressure due to the activity of plant roots, microbes, and fungi, which use oxygen as the terminal electron acceptor (electronegativity of oxygen = 3.5) in respiratory processes.

In submerged soil, within days or even hours, depending on the organism density, temperature, and fresh water supply, the oxygen partial pressure may fall to the limit of its detection. The lack of the electron acceptor oxygen causes a reduced redox potential in the soil solution, altering the mineral availablility for the plants (Ponnamperuma 1972; Li *et al.* 2006).

Root responses to oxygen deprivation

In roots, the lack of oxygen limits oxidative phosphorylation that cause a lack of ATP and resulting decrease in root growth rate (Drew 1997). Within several hours of anoxic conditions less efficient anaerobic respiration pathways are initiated that yield significantly less energy in the form of ATP (Perata and Alpi 1993; Drew 1997; Amedeo Alpi pers. comm. 2001) and produce ethanol and lactic acid as end products (Kennedy *et al.* 1992; Wigge *et al.* 1993; Pfister-Sieber and Brändle 1994; Kwast and Hand 1996). The root partly releases the high energy end products from anaerobic respiration since they are either toxic or reduce the pH of the symplast in the root tissue to unfavourable ranges. When measuring apparent root-zone respiration rate by means of O_2 or CO_2 exchange, rhizomicrobial respiration is always included. However, mere root respiration should be used for comparing the respiratory activity of plant roots under various soil conditions. During flooding, the apparent respiration rate decreases, but due to an enhanced percentage of microbial respiration, the decrease in mere root respiration rate is even more pronounced. The microbes benefit from the lack of oxygen since defense mechanisms of the roots are hindered, and the high energy compounds released by the roots support the microbe growth.

Limited ATP synthesis in plant roots under oxygen shortage has frequently been studied (Dry and Wiskich 1982;

Pradet and Raymond 1983; Crawford 1993), while few studies are available on the changes in the redox charge of co-enzymes (Morimoto and Yamasue 2007), which drives the redox reactions in cell metabolism. Co-encymes are oxidised in the aerobic respiratory pathway. In anaerobic respiration the oxidation takes place to a limited amount and different extent regarding the phosphorylated and non-phosphorylated forms (Perata and Alpi 1993; Pfister-Sieber and Brändle 1994; Liao and Lin 1995; Pezeshki *et al.* 1996). In many plant species, an alternative respiration path is present additionally to the cytochrome path. It is assumed that the alternative path acts as an overflow for accumulating reduced co-enzymes (Day and Lambers 1983). The residual oxygen uptake in the alternative pathway is supposed to be due to oxidation in the peroxisomes (Lambers *et al.* 1991). In the latter process, co-enzymes are oxidised, mostly the nicotinamide-adenine-dinucleotides, NAD(P).

Under anoxic conditions, a decrease in the oxidation rate of NAD(P) was found in isolated mitochondria (Wigge *et al.* 1993; Kwast and Hand 1996; Paul and Schneckenburger 1996) and *in vivo* (Hoffmann *et al.* 1993; Zude-Sasse *et al.* 2001; Morimoto and Yamasue 2007). The complex influence of the decreased NAD(P) oxidation on the plant due to oxygen deficiency is hardly investigated.

Citrus root stocks under oxygen deprivation

Greenhouse experiments were carried out on citrus root stocks to study short-term (max 5 h) and medium-term (max 54 h) plant responses. Side effects were largely excluded by rapidly inducing anoxia (within 30 s) by streaming the root cuvettes with gaseous nitrogen. CO_2 gas exchange of roots and leaves was measured with an infrared gas analyser in an open system. Total photosynthetic electron transport rate (ETR) was analysed by means of chlorophyll fluorescence kinetic analysis applying light saturation curve readings on dark-adapted leaves (Krause and Weis 1991). Discriminating analyses of oxidised $NAD(P)^+$ and reduced NAD(P)H were carried out after extraction of fresh plant tissue by enzymatic means recorded photometrically (Hoffmann *et al.* 1993; Zude-Sasse 2000). Anaerobic reduction charge (ARC) was calculated by $ARC=NADPH/(NADPH+NADP^+)$, while catabolic reduction charge (CRC) address $CRC=NADH/(NADH+NAD^+)$.

Root respiration apparently decreased immediately after starting the stress treatment. A reduced net photosynthesis rate (P_N) appeared within a period of a few hours that is consistent with former work on many plant species (Phung and Knipling 1976; Andersen *et al.* 1984; Peine *et al.* 1985; Smith and Ager 1988; Larson and Schaffer 1991; Vu and Yelenosky 1991; Beckman *et al.* 1992; Liao and Lin 1995; Pezeshki *et al.* 1996; Fernandez 2006; Li *et al.* 2007). The reduction in P_N is asssumed to be largely the result of stomatal closure due to enhanced ethylene or abscisic acid production and dissociation, resulting in reduced CO_2 uptake. However, the rate of ATP and NAD(P)H formation in the leaves constitute the driving force of photosynthesis (Giersch *et al.* 1980; Siebke *et al.* 1990; Gerst *et al.* 1994). A number of studies suggest a major influence of the degree of ADP phosphorylation regulated via the carbohydrate concentration of plants (Rao *et al.* 1990) and concluded that the rate of $NADP^+$ reduction regulates photosynthesis (Giersch *et al.* 1980; Siebke *et al.* 1990; Hanning and Heldt 1993; Wigge *et al.* 1993; Krömer 1995). In their model, the ARC and CRC determine the ETR in the two photosystems (PSI and PSII) in the chloroplasts. Subsequently, the increased redox potential of NADPH and corresponding deficiency of $NADP^+$ could limit the photosynthesis rate under anoxia ($PS\ II \rightarrow PS\ I \rightarrow Ferredoxin \rightarrow NADP^+$).

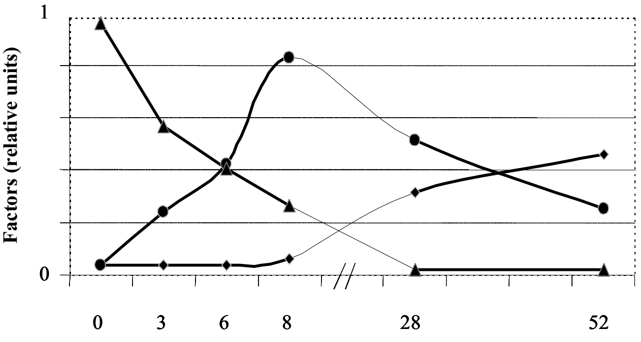

Fig. 2 Schematic of time-dependence of factors affecting net photosynthesis rate in short- and medium-term oxygen deficiency in the root zone after starting the stress treatment (time 0). ▲net photosynthesis rate, ● non-stomatal impact, ♦ stomatal impact (Zude-Sasse 2000).

The hypothesis of a non-stomatal influence was supported by empirical studies in citrus (Zude-Sasse and Lüdders 2000), mango (Zude-Sasse *et al.* 2001), and other plants (Peine *et al.* 1985; Kwast and Hand 1996; Morimoto and Yamasue 2007), i.e. oxygen deficiency leads to an increase of pyridine nucleotide charges. It was shown in citrus that total contents of NAD (1.2 nmol g^{-1} fw) and NADP (1.0 nmol g^{-1} fw) were unaffected by anoxia treatment in short-term. However, enhanced values of ARC and CRC were found. The ARC increased from 0.28 to 0.31, and the CRC from 0.12 to 0.31 resulting in free reaction enthalphy enhancement from -442.4 to -444.0 kJ mol^{-1}. Similarly enhanced values were found in the leaves. The higher values point to the decreased availablility of the oxidised molecules and diminished readiness for reduction in photosynthesis. Variations in ETR and efficiency of the photosystems (yield, y) (Krause and Weis 1991) indicated that the increased CRC and ARC appeared in parallel with a reduced y and light saturation of ETR at lower photon density (Zude-Sasse *et al.* 2001). Such finding might actually be a result of endproduct inhibition at the ferredoxin to $NADP^+$ electron transfer step. Within a few hours, both CRC and ARC in the leaves returned to the level determined prior to the treatment. Solely, in the roots the CRC remained high. In the medium term, net photosynthesis rate seemed to be limited by stomatal closure as indicated by the reduced ratio of internal/external CO_2 concentrations (**Fig. 2**).

Although the causal interactions of the redox charge of NAD(P) in roots and leaves remain unknown, such finding point to sensitive parameters in the plant response and, consequently, to possible advanced methods for monitoring the plants in the production: Drought or osmotic stress (Neumann 2008; Storey and Walker 1999) and oxygen shortage often result in similar plant symptoms becoming visible when plants are already severely damaged. While sensors for environmental conditions as well as the plant response to drought stress exist, the influence of oxygen deficiency on the plant is hardly approached. Particularly, short-term response to anoxia might be detected by means of the ETR, y, and NAD(P) charges.

Long-term (several days) response of plants to anoxia is even less investigated. Here, two cases must be distinguished: (1) Plants adapt to anoxia and survive, and (2) plants are not able to adapt and die. Adaptation to anoxia is, however, often accompanied by significant growth and yield reductions and therefore detection in due time by non invasive measures would be of large advantage. In the second case, plants are irreversibly damaged very quickly and here an extremely rapid plant response measurable by means of feasible method is requested.

Detecting anoxia in the root zone

Soil (or substrate) parameters can be measured and examined in relation to anoxia. Recently optical sensors were developed for the measurement of gaseous (Cao and Duan 2006) and dissolved oxygen. Such sensors have several advantages compared to the conventional Clark-type amperometric oxygen electrodes which consume oxygen and therefore need a specific flow rate of the soil water or nutrient solution. Paramagnetic devices provide accurate readings, but are expensive. Oxygen sensors could be applied to detect unfavourable conditions in the root zone. The critical oxygen partial pressure is determined by the oxygen consumption of the roots, mainly by the respiratory activity, tissue resistance, and radial distance from the rhizodermis to the mitochondria. However, it is difficult to estimate the effect of the oxygen concentration on the plant due to non-uniform distribution of the oxygen concentration in the root environment and in the tissue as well as the apparent adaptations of the roots. Moreover, the oxygen demand of the roots and thus the critical oxygen partial pressure in the root environment depend also on the photosynthesis affecting the root respiration by providing the assimilates.

The same difficulties appear when considering the soil water content. Suitable sensors for the measurement of the soil water potential, such as tensiometers, provide data on the available water, but in submerged soil the saturation of the soil is more interesting. The soil water content can be measured by various methods, mostly calibrated on the gravimetrically measured water, such as lysimeter, Bowen ratio, scintillometer, eddy correlation, and time domain reflectometry. Methods applied for spatially resolved sensing, such as geo-electrical and electromagnetic induction sensing, or spectroscopic approaches in the near infrared wavelength range are available and provide spatio-temporal data on the soil conditions. Enhancing the observation level by means of remote sensing (microwave, radar, polarized light, spectroscopy in the visible, shortwave near infrared up to thermal infrared wavelength range) enables landscape assessment (Verstraeten *et al.* 2008), but apparent signals might be pertubated due to dense canopies. Soil- or substrate water content, regardless the observation scale, itself are, however, less suitable to characterise anoxia in the root environment in relation to its effect on plant growth, but provide information to distinguish between water deficit and oxygen deficiency when a reduction of growth, photosynthesis rate or any other crop parameter is detected.

The most specific indication of oxygen deficiency in the root environment is the strong decrease of the aerobic root respiration (Lambers *et al.* 1991). Root respiration may be derived from the CO_2 efflux from the soil or the substrate. Here, the possible sources of CO_2 in the root environment need to be considered. Kuzyakov (2006) distinguished five main biogenic sources of CO_2 efflux from soils: root respiration (1), microbial decomposition of rhizodeposits (2) and dead plant remains (3), microbial decomposition of soil organic matter in root or plant residue affected soil (4) and in root-and-plant residue free soil (5). The processes feeding the sources (4), (5), and partly also (3) are passing off slowly (Kuzyakov 2006) so that their contribution to the total CO_2 efflux from the soil or substrate may be neglected when measuring root respiration. In contrast, sources (1), (2) and partly (3) contribute significantly to the CO_2 efflux rates from soils or substrates. Presently only destructive and isotopic methods are available to distinguish between root (1) and rhizomicrobial (2) respiration (Kuzyakov and Larionova 2006). In any case, a low total CO_2 efflux from the soil or substrate provides an indicator for oxygen deficiency in the root environment. Most often two non-destructive methods are applied to measure the CO_2 efflux from the soil. The classical chamber method, where the CO_2 flux is measured by a gas analyser, yields reliable data (Davidson *et al.* 2002). Despite its high purchase, installation and maintenance costs, nowadays eddy covariance techniques to measure the soil CO_2 efflux has been temporarily applied (Baldocchi 2003). Tang *et al.* (2003) proposed the estimation of CO_2 fluxes based on continuous measurements of the soil CO_2 profile. Measuring profiles is less expensive than the use of chambers. However, difficulties may be expected when the soil is waterlogged or flooded.

In hydroponic systems or containers, the measurement of the root respiration is more difficult. The CO_2 respired by the roots may diffuse out of the substrate in the gaseous phase, but also leave the substrate dissolved in the drainage solution. The substrate volume captured by the roots represents a small fraction of the ground area, making the application of eddy covariance and substrate profile techniques nearly impossible. Despite the problem of the dissolved CO_2 in the drainage solution, imbedding the substrate in a chamber seems to provide a suitable method to measure the root respiration rate. This method is non-destructive, but invasive: The environment of the substrate in the chamber differs from the "natural" environment in terms of wind speed, CO_2/O_2 concentrations and CO_2/O_2 gradients at the substrate surface. These effects must be evaluated.

Methane production in submerged soil (Ponnamperuma 1972; and e.g. Megonigal and Guenther 2008) plays a major role in greenhouse gas assessment, but does not provide sensitive data for measuring the plant responses in the short-term.

Measuring directly on the plant, the water transport can be measured using porometer, and more simple temperature of leaves, stem diameter, xylem sap flow, as well as D_2O. Results would provide valuable data for detecting drought stress (Fernandez *et al.* 2008), while the response due to anoxia appears with a time-lag and with similar symptoms resulting in non-helpful signals for adjusting production measures.

The oxygen concentration in the plant tissue may also be considered as an indicator for anoxia. Recently, techniques based on oxygen-sensitive phosphorescent microbeads have been developed to measure absolute intercellular oxygen concentration in plants (Schmälzlin *et al.* 2005). Such minimal-invasive techniques were preferably used to characterise the metabolic activity depending on internal oxygen concentration in seeds and potato tubers (van Dongen *et al.* 2004). Likewise soil or substrate oxygen concentration, tissue oxygen concentration cannot be expected to be homogensously distributed in the roots, which makes the establishment of critical values of the oxygen concentration complex and certainly these values also depend on several environmental conditions as well as on the status of the plant.

Detecting anoxia from the plant responses

Beside or in addition to soil or substrate attributes the plant's response to anoxia should be evaluated. Anoxia in the root environment results in a strong decrease of shoot and root growth. Relative stem elongation is probably the easiest to measure plant parameter related to growth. Stem elongation can be measured simply by using a ruler or more sophisticated on-line using linear displacement position sensors (Oki and Lieth 2004). Measured stem elongation can be related to a standard elongation described for many crops as a function of environmental conditions – most often of air temperature by means of Arrhenius approach (Tijskens and van Kooten 2006). Stem growth decreases under oxygen shortage, but such effect can be caused by other stress factors including drought and high salinity and is therefore not suitable for a specific diagnosis of anoxia in submerged soil. Moreover, in some plants, such as rice, increased stem elongation is a mechanism of anoxia tolerance under flooding and submergence (Gibbs and Greenway 2003).

The same problems appear when leaf area is considered. Leaf area can be derived from simple linear non-destructive measurements of leaf length or width (Schwarz and Kläring 2001), or using remote sensing (e.g. Elwadie *et al.* 2005). Measured leaf growth may be related to standard leaf growth calculated by a model (Marcelis *et al.* 1998; Lee and Heuvelink 2003). Inhibition of leaf growth was often observed under oxygen deficiency, but leaf growth is also reduced under osmotic stress. The measurement uncertainty of leaf area of adult plants is relatively high compared to the leaf area increment in the short periods. Remote sensing methods allow to separate leaf growth and stress response, if wavelength dependent data are considered.

Shoot and root mass are affected by oxygen deficiency in the root environment. Several methods to measure plant mass non-invasively are available. Balances can be used to follow the mass development of plants grown in containers, if the mass differences can be reasonably attributed to the plant growth. However, evapotranspiration and irrigation limit a high temporal resolution of the measurements. In the 1950s radioisotopes were tested to measure the mass of sugar cane canopies by analysis of the absorption of the gamma-rays by the plants (Burr *et al.* 1956). Originally this gamma-scanning technique was applied to uniform canopies, such as cereals, but nowadays vegetables can also be measured with reasonable accuracy (Gutezeit 2000a). Gutezeit (2000b) used this technique for irrigation control. In an experimental approach plants were irrigated, when its non-invasively measured growth rate was significantly lower compared to the well-irrigated control. Remote sensing based on reflectance and transmittance measurements also may be used to estimate the green biomass of a canopy (Meron *et al.* 2000; Hansen and Schjoerring 2003). Additionally, laser-scanning techniques were applied in citrus for measuring the canopy density (Wei and Salyani 2005; Zaman *et al.* 2006b). Some of this equipment provides very accurate measurements of mass increment for short periods of one day. However, it must be considered that they capture all variations in canopy mass due to changes in the water status of the plants. When using mass increment measurements for irrigation control, an additional measurement is necessary to distinguish between an effect of water deficit and water logging. This may be a rough measurement of the soil or substrate moisture.

Spectral-optical analyses of leaves

Reflectance readings - Adaptation to oxygen deficiency always includes coping with an energy crisis due to the lack of the terminal electron acceptor in the respiratory chain as well as protecting the cell against the reducing pressure (Zude-Sasse *et al.* 2001; Gibbs and Greenway 2003). The latter may cause changes in the xanthophyll cycle resulting in variation in the carotenoid composition. Changes in the related carotenoids zeaxanthin, violaxanthin, and antheraxanthin, were reported earlier as measurable by means of reflectance spectra and were recorded in remote-sensing or in contact with the leaf under various stress conditions (Peñuelas *et al.* 1995; Gamon *et al.* 1997), but were hardly studied under anoxic conditions. Adapted indices or multivariate methods for processing the leaf spectra may provide, however, relevant data. For instance, the ratio of reflectance intensity at 555 nm and 450 nm changes due to anoxia in the root zone of citrus (**Fig. 3**), maybe pointing to a method that detects a plant response which is specific to anoxia.

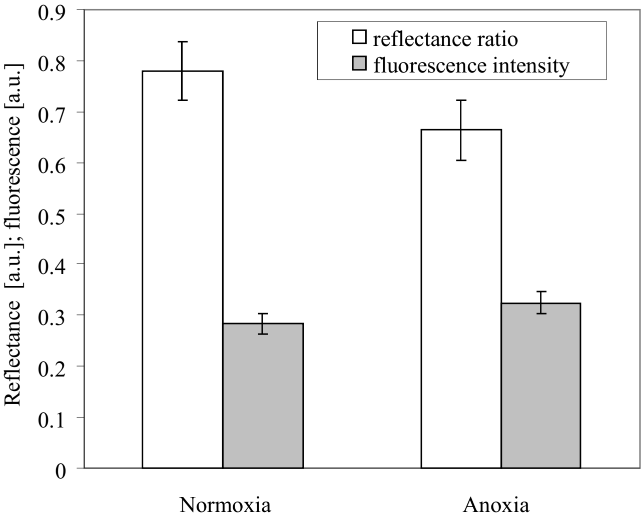

Fig. 3 *In-situ* readings carried out on citrus leaves (*Citrus reticulata*). Leaf diffuse reflectance is presented as the ratio of reflectance intensities at 555 nm and 450 nm. Reflectance values were recorded with a handheld photodiode array spectrophotometer (PA1101, CP, Germany) equipped with LED serving as light source and calibrated with white reference (spectralon, Labsphere, USA). The leaf apparent fluorescence signal was measured with excitation at 337 nm and emission at 460 nm at a time gate of 6-8 ns applying a fluorimeter equipped with glass fibre (LF401, I.O.M., Germany). Data are expressed as normalized values between 0 and 1. Bars represent SE.

Changes in chlorophyll (Carter 1994) and flavanoids are detectable by means of reflectance and by fluorescence analyses and provide data on the plant stress response (Moran and Moran 1998; Merzlyak *et al.* 1999; Hagen *et al.* 2006). Former experiments indicated that these approaches are suitable to distinguish between adaptation and irreversible damage of plants. A specific response to anoxia can hardly be expected. Reflectance measurements based on remote sensing may, however, be used to estimate integrated stress responses of the canopies (e.g. Peñuelas *et al.* 1998; Roberto *et al.* 2002).

As a result, reflectance readings in the visible and up to infrared wavelength range, changes in the pigment profiles and temperature and emission coefficients can be measured (Möller *et al.* 2007). In shifting the observation level closer to the leaf, imaging techniques can be used for measuring the stomata aperture, which provides an apparent signal for different plant response to environmental and endogenous stress (e.g. Dzikiti *et al.* 2007). However, again it is questionable whether this signal is specific to anoxia.

Chlorophyll fluorescence - Besides the analysis of reflectance intensity due to absorption and scattering characteristics of the canopy, the re-emission of radiation can be considered. Fluorescence is a highly specific phenomenon appearing in molecules, which possess electrons that can be excited from the ground state (S0) directly or over an intermediate to their excited singlet state (S1*) when absorbing radiation and return to the ground state again, while re-emitting radiation. Chlorophyll shows fluorescence with high quantum yield in the red wavelength range. First methodology development began with much more precise techniques by measuring time-resolved chlorophyll fluorescence with nano second resolution (Brody and Rabinowitch 1957). In parallel, research was carried out leading to the discovery of the Kautsky effect (*c.f.* Krause and Weis 1991). During the last few decades, chlorophyll fluorescence analysis has become an accepted method in plant physiology, using appropriately adapted instruments.

Chlorophyll fluorescence kinetic analysis (Kautsky and Hirsch 1931; Krause and Weis 1991) has been applied in several approaches for measuring the activity and capacity of photosystem II under various stress conditions (Lichtenthaler and Rinderle 1988) and is used in applied research in the fields of precision agriculture (Lüdeker *et al.* 1997) as well as fruit decay monitoring (Nedbal *et al.* 2000). Under anoxia in the root zone, the ETR and y provide useful parameters that can be analysed non-invasively, sometimes leading to remarkable differences in control and stressed plants (**Fig. 4**). The differences between and within the leaves, already before the treatment are, however, also noticeable. Spatial distribution of the electron transport rates in leaves and the influence of leaf development stage need to be taken into account when considering a feasible application of the method.

Instruments for measuring the spatial distribution of the chlorophyll fluorescence of plant leaves are available for the past since few years from the companies, PSI (Czech Republic) and Walz (Germany). Furthermore, recent efforts in the field of laser development result in new light sources with low weight and robust design (Sumpf 2009). Promising results can be obtained with new laser light sources serving for exciting electrons of molecules under interest, e.g. for measuring the fluorescence appearing in the blue-green in addition to the red wavelength range.

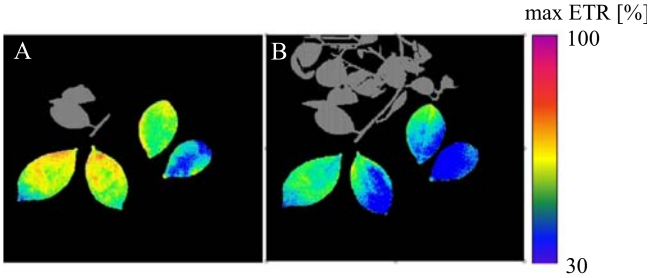

Fig. 4 Electron transport rate (ETR [µmol m^{-2} s^{-1}]) measured with a pulse-modulated imaging system for analysing the chlorophyll fluorescence kinetic (FluorCam FC 800-O/1010, PSI, Brno, Czech Republic). Presented in normalized false colour coding [0-100%] of four leaves before (**A**) and after 36 hours anoxia (**B**) in the root zone of citrus rootstock *Citrus volkameriana* in a greenhouse. The maximum ETR was derived at the inflection point of regression curve of light saturation curves measured after dark adaptation with increasing photon flux density (max. 600 ± 30 µmol·m^{-2}·s^{-1}).

Blue-green fluorescence **-** Fluorescent compounds fluorescing in the blue-green wavelength range were rarely investigated. However, the technique of laser-induced fluorescence spectroscopy (LIFS) might provide an additional non-invasive tool to address plant compounds. Lichtenthaler (Buschmann and Lichtenthaler 1998) and co-workers used a fluorescence imaging system equipped with laser light sources to obtain non-invasively images of leaves to detect changes in the blue-green fluorescence. The apparent fluorescence intensities are the combination of the fluorescence of a variety of native fluorescent and quenching compounds in the plant. Such molecules are, e.g., chlorophyll a and b, and chlorophyll precursors, riboflavin, pyridine nucleotides (NADH and NADPH), ferulic acid derivatives and others (*c.f.* Buschmann and Lichtenthaler 1998; Lakowicz 2006). In extracts, it is possible to perform quantitative fluorimetry according to the following equation:

$$I_F = k \cdot \Phi \cdot I_0 \cdot \left(1 - 10^{[-\varepsilon \cdot l \cdot c]}\right)$$

where I_F is the apparent fluorescence intensity, k is a geometric instrumental factor, Φ is the quantum yield (photons emitted/photons absorbed), I_0 is the intensity of the excitation source, epsilon is the wavelength-dependent molar absorption coefficient, l is the path length and c is the analyte concentration. However, the pH-value of the extract determines the redox status of the molecule and strongly influences the energy level of the relevant electron. The interaction of the dipole momentum of the molecule under question with the dipole moment of the solvent or the tissue matrix, when measuring an intact plant organ, including all possible interactions as direct response on the solvent molecule and solute molecule, appearance of dipole moment changes due to interaction of solution and solute molecule, as well as static quenching and reabsorption effects, make such approach difficult.

That such effects severely affect the fluorescence interpretation *in-situ* is obvious, but sometimes such uncertainty appears partly as an advantage: This is the case for NAD(P), where the oxidized NAD(P)$^+$ shows no fluorescence, while the reduced form NAD(P)H fluoresces due to appearing stable electron delocalization at the nicotinamide ring. For example, a nitrogen laser emitting at 337 nm can be applied (LF401, I.O.M., Germany) for exciting NAD(P)H (pH = 8.4) resulting in fluorescence at characteristic band passes.

The molecule conformation and charge of pyridine nucleotides were already measured in isolated mitochondria by the molecular fluorescence intensity (Brody and Rabinowitch 1957; Siffel *et al.* 1991) and fluorescence relaxation time (Ladokhin and Brand 1995; Paul and Schneckenburger 1996). Studies on the influence of anoxia on the redox charge of NAD(P) by means of fluorescence are promising, but still rare.

When directly measuring on the plant tissue, a broad peak is visible in the blue-green range due to mainly the polyphenol fluorescence. The difference of the spectral profiles might be used for calibrating on the NAD(P)H. Resulting wet-chemically methods might be partially replaced by non-destructive *in-situ* measurements. Recently, technical solutions became available for measuring NAD(P)H in intact animal tissue (e.g. Pogue *et al.* 2001). The achievement of such application in plant organs appear more difficult due to phenolic compounds masking the NAD(P)H fluorescence. Time-resolved laser-induced fluorescence might solve such problems by providing additional information on molecules` fluorescence relaxation time.

Fluorescence relaxation is altered due to the influence of different environmental conditions and is therefore *in-situ* subject to changes. Such information may enable, however, the discrimination of NAD(P) fluorescence (0.4 ns in methanol at pH = 8.2) from chlorophyll a fluorescence (5.9 ns in methanol) and the overall polyphenols' fluorescence. Preliminary results achieved when investigating reduced oxygen partial pressure in the root zone of rooted citrus cuttings support such a suggestion (**Fig. 3**).

Furthermore, protein marker genes emitting green fluorescence might lead beyond the border of molecular sensing to gene expression analyses during the processes (Chong *et al.* 2007), such as targeted by means of real-time PCR. However, such approaches require the implementation of indicator plants set in the orchard.

Advantages and bottlenecks of non-invasive measurements

Non-invasive measurements (**Table 1**) carried out temporarily during the entire production period may indicate in due time harmful oxygen concentrations in the root environment or plant tissue and enable the farmer to take action to avoid growth and yield reductions. Some of these measurements are easily to perform in a plant canopy, others require a laboratory and are therefore not suitable for practical use. Only few methods were evaluated to detect anoxia. In any sensor-based method two main questions need to be solved before practical application:

1. Most stress factors such as anoxia, salinity or drought stress result in similar plant responses, which make it difficult to derive the necessary measure. Dual measurements of the soil properties, which indicate the most likely stress factor and the plant parameter, which estimates the stress level, may overcome these difficulties.

2. All available studies compare the response of anoxia treated plants to that of standard plants well supplied with oxygen. The signal of the standard, however, is specific to the genome, and depends on many factors, such as age and position of the leaf. Providing a threshold is the most difficult to obtain task for practical applications.

Besides the problems related to sensors, plants of the same species and cultivars, which adapt to oxygen deficiency in the root environment and survive, respond differently compared to those which die. Processes, such as photosynthesis and chlorophyll fluorescence kinetic of the leaves of adapted plants, may not be negatively affected, although overall growth is significantly reduced. Adaptation to anoxia is, however, accompanied by changes in the metabolism (Gibbs and Greenway 2003), resulting in different leaf content of chlorophyll, carotenoids or polyphenols. These pigment contents may be evaluated by means of spectral absorption coefficients, which favor this method with regard to the detection of an anoxia specific plant response.

Table 1 Examples of non-invasive measurements on substrates and plants to detect and evaluate oxygen deficiency in the root environment (extract from the literature cited in this section).

Plant or substrate characteristic	Non-invasive measurement devise	Characterised attribute or process	Advantages	Bottlenecks
Oxygen in the root zone	Galvanic, polarographic, optic, or paramagnetic	Substrate oxygen concentration	Indicates anoxia as stress factor	No information on the stress level in the plant
Oxygen in the plant tissue	Optical detection of microbeads	Tissue oxygen concentration	Indicates anoxia as stress factor	Large variation in the tissue, critical values unknown
Soil moisture	Tensiometer, time domain reflectometry	Soil water content	Distinguishes between water surplus and deficit	No information on the stress level in the plant
Substrate O_2/CO_2 exchange	Open chambers using infrared gas analyzer, eddy covariance technique	Root respiration	Indicates limitation in root respiration	Does not distinguish between the sinks/sources of O_2/CO_2 -plant roots or microorganisms
Stem length, leaf area, root length	Ruler, linear displacement position sensor, remote sensing, rhizotrons, X-ray computed tomography	Stem leaf and root growth	Partly very simple methods	Slow and unspecific plant response, root growth very difficult to obtain in a canopy
Mass	Balances, gamma-scanning, X-ray scanning, laser-scanner, impedance spectroscopy, RAdio Detection And Ranging	Growth, water content, transpiration, shoot/root ratio	Accurate measurement of above ground plant mass of a larger canopy possible	Slow and unspecific plant response, requires very sophisticated equipment, root mass very difficult to measure
Leaf gas exchange	Open or closed chambers using infrared gas analyzer or photochemical sensor	Photosynthesis, transpiration, stomata conductance	Standard method, provides many information related to anoxia	Depends on leaf position on plant, leaf age and adaptation to various environmental conditions, unspecific plant response, anoxia adapted plants may not respond
Chlorophyll fluorescence kinetic	PAM, imaging	Efficiency of photosystem II, light saturation	Rapid standard method	Difficult to find a robust indicative parameter, anoxia adapted plants may not respond
NADP fluorescence	Laser-induced, time-resolved fluorimeter	Reduced NAD(P)H	Rapid	Unknown uncertainties due to variation within and between plants
Spectral remittance of leaves	Spectrophotometer, photo diodes	Chlorophyll, carotenoids, polyphenols contents	Rapid with the potential to distinguish among plant responses to different stress factors	Unknown uncertainties due to variation within plants and with environmental conditions
Canopy reflectance	Scanning spectrophotometer, photo diodes, thermography, hyperspectral cameras	Chlorophyll, carotenoids, polyphenols per area, transpiration per area	Potential of on-line measurements on a larger canopy	Expensive, specific software to be developed, error-prone with changing environmental (light) conditions

PRECISION HORTICULTURE APPROACHES FOR THE ASSESSMENT OF THE QUALITY OF *CITRUS* FRUIT

Quality of citrus fruit

If the cultivar and rootstock combination is planted, the potential fruit quality is set (Fallahi and Rodney 1992; Castle 1995; Barry *et al.* 2004a, 2004b). However, in the production, endogenous plant factors, production measures, the microclimate, and soil conditions have a major effect on the fruit quality. Young and vigorous trees often provide an unpredictable variation of fruit quality due to unbalanced phytohormone levels. In mature trees, the variation of fruit quality within the canopy becomes more predictable. The carbohydrate production in the photosynthetic active leaf area and allocation appears in relatively short-distances. Also, suitable pruning measures balance the vegetative and generative growths and corresponding phytohormone levels. Sun-exposed sectors of the canopy with high photosynthetic active photon flux density produce fruits with enhanced sugar contents in comparison with fruits grown in shaded inner sectors of the canopy. However, it might be interesting to check such distribution in certain plant development stages as well as after pruning measures, for obtaining an objective decision basis regarding the number and protocol of selective harvestings.

Additionally to the endogeneous factors and the production measures, the microclimate and soil conditions influence the fruit development and resulting fruit quality. Variation in the fruit maturity stage between the canopies is influenced by temperature and moisture, both affected by disturbances of the boundary layers due to air movement triggered by wind velocity and air pressure gradients, as well as radiation density. Soil type and texture provide the substrate for the supply of minerals, water, and oxygen. The site-specific monitoring of the plant response to the different factors and resulting effects on the fruit quality is presently taken into account on a subjective basis, if the grower knows his/her orchard and the variation appearing. The major reason for this uncertain approach is the lack of feasible methods for analysing variations in crop quality in the orchard (**Fig. 1**).

The current destructive methods to detect citrus fruit quality recognize the parameters: soluble solids content (SSC), acidity, and juiciness. In the production, the fruit maturity on trees is most often evaluated with respect to the SSC. For the measurement a fruit juice drop is subjected to a refractometrical analysis. The refractometer measures the total reflection at the boundary layer between glass, serving as the carrier for the juice drop, and the fruit juice. The refractive index (Snellian law) obtained is calibrated on pure sucrose concentration. The apparent SSC [%Brix] is mainly influenced by carbohydrates, organic acids, carotenoids, and free amino acids. A better impression of the fruit taste is provided by the ratio of SSC to acidity (*c.f.* Nagy 1996). However, the SSC is a feasible parameter for determining the optimum harvest date.

During citrus fruit development, the SSC increases until a steady-state is reached. At this point fruits should be harvested to avoid possible crop damage due to unfavourable weather conditions, like high precipitation rate, frost, and high radiation. A major disadvantage of the method results from the destructiveness of the measurement, since random spot readings are not suitable for fruit monitoring, and in practise only a low amount of fruits will be analysed. The high heterogeneity of the fruit quality within the canopy often demands selective harvesting. But do we know enough on the different cultivars to determine the efficient number of harvests? Furthermore, can we expect a significant variance in fruit quality in different sites of the orchard? More importantly, where should the harvest manager will send the harvest team in the first place? It seems that we presently rather often guess, while loosing money either due to expensive harvests or by means of down grading of the crop due to high heterogeneity. With new methods such questions might be approached by means of non-destructive fruit monitoring based on near infrared spectroscopy (NIRS).

The application of near infrared spectroscopy for crop monitoring on the tree

Monitoring the crop of the trees is essential to support the decision-making in production and harvesting processes (Sanders 2005; Caixeta 2006). The prediction of the crop yield (Iwaya *et al.* 2005; Ye *et al.* 2008), possible damages (Zaman *et al.* 2006a) or quality of the crop have been approached by means of different techniques, such as colour vision systems (Annamalai *et al.* 2004), hyperspectral cameras, laser-scanning technique (Wei and Salyani 2005) and ultra-sonic readings (Schuhmann and Zaman 2005; Zaman *et al.* 2006a), spectroscopic analyses in the visible and near infrared (NIR) wavelength range (McGlone *et al.* 2003). Depending on the observation level, correlation of measured parameters are applied, such as canopy density analysed with remote spectral analyses, and can be used for yield estimation (Iwaya *et al.* 2005; Ye *et al.* 2006, 2007, 2008). Shifting the observation level closer to the fruit, – while increasing the percentage of the signal that is directly altered by the molecule under question – particularly visible and NIR spectroscopy is suitable to obtain data on the internal fruit properties. In the visible wavelength range, carotenoids (Zude *et al.* 2007) and nutritional valuable flavanoids (Tripoli *et al.* 2007; Wulf *et al.* 2008) might be detected in the future. Main research in the NIR wavelength range for monitoring crop quality has been conducted for two decades, and results point to feasible applications in the process management of citrus.

In agriculture NIRS has been applied to remote sensing for gathering information on the vegetation and soil properties (Knipling 1970). Non-destructive analysis of agro-food products has been studied since the sixties. Practical use was made since approximately 1980, when alcohols, sugars, proteins, fatty acids contents as well as structural changes in the conformation of starch, water cluster and protein were addressed. Presently, the NIRS is intensively used for determining the cereal quality with respect to protein, carbohydrate and water content. The market price of grained cereals is based on this method, which is supported by an authorized data bank system for calibration and secure data saving purpose.

Molecular overtones can be identified as absorption bands in the remittance and transmittance spectrum in the NIR wavelength range captured by fast diode array sensors. In contrast to the characteristic absorption peaks of pigments in visible spectroscopy caused by excitation of electrons, near infrared spectra of molecules' vibrational overtones show low intensity and broad bands due to overlapping vibrational modes. An assignment of peaks to individual molecule vibrations is normally not possible for calibrating on the soluble solids content or specific sugar contents. Due to the high water content of fresh fruit the absorption by functional groups such as –OH may result from water as well as carbohydrates. As result, it cannot be expected that robust calibrations working on various cultivars will be gained in the future (Sanchez *et al.* 2003; Golic and Walsh 2006; Nicolai *et al.* 2007), but even data with high measuring uncertainty might support decisions in the horticultural practise.

Since 1970, enhanced research emphasis has been carried out on developing wavelength-based or whole-spectra analytical methods for non-destructive determination of SSC, sugars and acids. The specific fingerprint of molecules are figured out qualitatively by developing a mean spectrum with adjusted confidence interval, which represents the sample's typical absorbance properties appearing in the NIR wavelength range. Most often linear methods are applied for quantitative analyses. A calibration data set is gathered that serves as the calibration matrix referenced by the related matrix representing the true values. The characteristic matrix of weighing factors is calculated using different decomposition methods to achieve smaller data sets, while the error limits the accuracy of the calibration model. For instance, the sugar content of unknown fruits can be predicted by means of the fruit spectra. Taking into account an extended spectral range in the data processing, e.g. up to 1700 nm, will normally improve the predictive capability of the model, since the molecules' fingerprint can be addressed by more variables responding characteristically due to additional molecules' vibrational overtones of lower orders captured in this wavelength range.

A detailed review of NIR physics for agricultural and food products was presented by Birth and Hecht (1987). Using the near infrared wavelength range up to 1700 nm, the SSC can be determined in intact produce (e.g. apple, banana, forages, kiwifruit, mango, melons, peach and potato) with $r^2 \geq 0.93$ and standard error of calibration (SEC) < 0.5 %Brix. However, when using only the reduced range from 800 to 1100 nm sufficient results can still be expected (Chen and Nattuvetty 1980; Bellon *et al.* 1993; Slaughter 1995).

Product monitoring using non-destructive NIRS might be particularly useful in citrus, since such readings can provide objective data on the fruit quality and maturity stage, regarding the fruit SSC [%Brix]. Miyamoto and Kitano (1995), as well as Kawano (1994), published early approaches in SSC prediction of citrus fruit using NIRS and PLS (partial least squares) regression analysis for calibration. Many research groups adapted the methods to citrus and added aspects on the histological aspects in citrus (Fraser *et al.* 2003; McGlone *et al.* 2003).

Different research groups (mainly in Japan, New Zealand and the United States have started to investigate the potential of spectral-optical methods for determining the maturity and quality of fruit (Olsen *et al.* 1969; Iwamoto *et al.* 1995). Satisfactory accuracy in practice was achieved by using the recently developed photodiode array spectrophotometers (400–1100 nm), which are fast, robust, shock- and dust resistant and available at a reasonable price. Since 2000, new sorting lines are commercially available, which appear as an innovative milestone in marketing of produce additionally to the established machine vision systems for citrus grading (e.g. Miller and Drouillard 2001; Aleixos *et al.* 2002). Commercial sorting lines are presently manufactured by companies world-wide (e.g. http://www.aweta.nl; http://www.greefa.nl; www.sacmi.it), working on the basis of spectral analysis in the visible and in the near-infrared wavelength range. Food preference is based on a complex system, involving cultural and sociological factors determining the product parameter valorized in the market. The weighing technique described is intended to complete the add-value processes by means of addressing the specific markets with optimum fruit quality properties. For example, in Japan or at citrus production sites producing for the Japanese market, the return-on-invest regarding such sorting lines can be reached after 2 or 3 years. Approximately 6 fruits per second can be graded on the fruit SSC with such technique. Further weighing parameters are under development. For example, the detection of internal tissue browning or even fruit acidity and particular sugar contents will be addressed in the future. However, such precise prediction is only possible, when an extended wavelength range up to 1700 nm is utilized (McGlone *et al.* 2003; Zude *et al.* 2007).

While sorting lines equipped with NIR sensor are commercially available nowadays, devices for measuring directly on the tree are still under development. Experiments with grapefruit were undertaken to evaluate the method

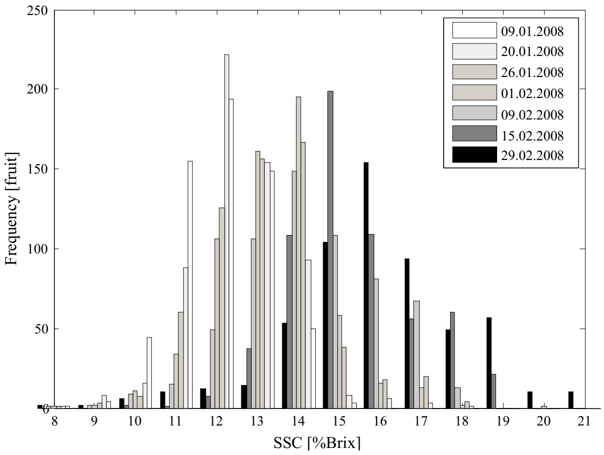

Fig. 5 Histogram of the quality change of citrus fruits (*Citrus reticulata* var. N59) grown in season 2007/2008, CA, U.S.A. Non-invasive spectroscopic readings started in January 2008 and were carried out until the fruits were harvested measuring 1 fruit visually representative per tree (n_{total} = 6300) in a commercial orchard.

using inline and hand-held NIR units with the fruit subsequently juiced to obtain a reference based on refractive index measurement of the fruit SSC. Testing prototypes of the devices, a linear regression showed highest coefficient of determination, r^2 = 0.67, in grapefruit at adjusted temperatures (Miller and Zude 2004). However, the temperature influence (Kawano 1994; Wülfert *et al.* 1998; Sanchez *et al.* 2003; Golic and Walsh 2006) and varying lightening conditions in the outdoor readings and working on specific wavelengths can increase the measuring uncertainty of the non-destructive analysis, if not taken into account in the calibration (Roger and Bellon-Maurel 2000; Sanchez *et al.* 2003; Golic and Walsh 2006; Nicolai *et al.* 2007; Zude *et al.* 2008a). Also, further logistic attempts to calibrate on the native fruit compound under question are under development (Fukhansky *et al.* 1993; Vogelmann 1993; Tsuchikawa and Hamada 2004; Zude *et al.* 2008b; Torricelli 2009).

Applications of non-destructive methods in the fruit production have been shown that the monitoring of individual fruit during its development on tree can lead to data which are easier to interpret since they do not represent the stochastic variability of heterogeneous fruit samples (Herold *et al.* 2005). Repeated from earlier readings (Zude *et al.* 2008a) non-destructive fruit monitoring was carried out weekly (n = 7) in season 2007/2008 with 6300 fruit readings for monitoring the development and spatial distribution of fruit SSC. On the first measuring date, the SSC ranged from >8 to <15 %Brix with mean 11.8 %Brix was found, while on the last date, the highest frequency of fruits with average 16.4 %Brix appeared (**Fig. 5**). Objective data on the fruit development in the orchard may help to schedule measures.

The single fruit shows three directional gradients in soluble solids content (Peiris *et al.* 1999), which diminishes gradually after harvest. More important for the harvest management, variation appears also between fruits within the canopy. Fruits from the inner sections of the canopy develop differently from fruit grown in exposed positions (Kender and Hartmond 1999). Most often, the fruit SSC increased from inner parts to exposed parts of the growing location in the canopy (**Table 2**). The variance within the canopy was reduced in trees planted in low distances (0.5 m × 2.0 m) with pruning measures for obtaining small trees compared to high density canopies planted with high planting distance (2.0 m × 4.5 m) in the field. Such data can help to decide on the planting system and pruning strategy, as well as provide objective information on the number of selective harvestings necessary in the production site.

Table 2 Mean and standard deviation (SD) of *Citrus reticulata* var. 'N59' fruit (n=600) grown in 2 locations, distinguished between the fruit growing position in the canopy by means of a handheld spectrophotometer unit as described in Zude *et al.* (2008). Three fruits per tree were measured in a commercial citrus production site in California, USA. Dense canopy refers to intensive, but conventional farming practise, while low-density canopy refers to a production system with canopies pruned similar to the spindle concept applied, e.g., in apple production.

	exposed	middle	inner part	Total SD
Dense canopy, bushy tree form, planting distance 2.0 m • 4.5 m				1.05
mean	11.32	11.15	10.51	
SD	0.94	0.94	1.09	
Low-density canopy, spindle-like tree form, planting distance 0.5 m • 2.0 m				0.93
mean	11.14	10.86	10.35	
SD	0.93	0.83	0.87	

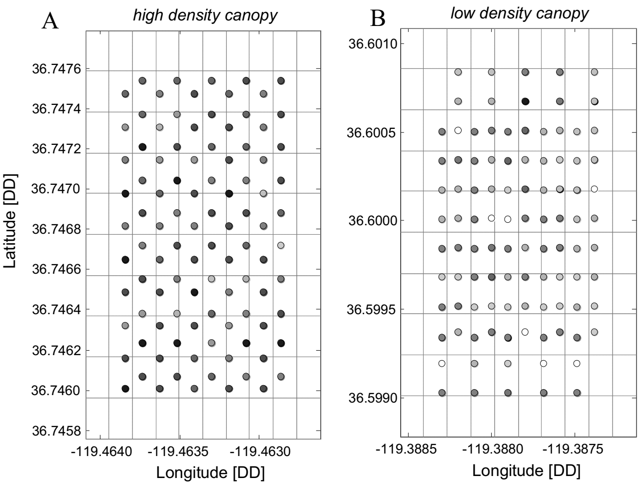

Fig. 6 Spatially resolved standard deviation (grey levels representing the range from 0.4 to 1.4) of soluble solids content (SSC) of fruits (*Citrus reticulata* var. N59) grown in inner, middle, and exposed sections of big and dense canopy (A) and small, spindle-like canopy (B). Measurements (n = 600) were carried out in January 2008 within one day and plotted based on geodetic data (WGS84-format).

An economic assessment of new smaller tree sizes with appropriate pruning (Lee and Rosa 2006) would be required to evaluate the benefit of small tree size on the number of necessary selected harvestings (Sanders 2005), taking into account the drawbacks of high investment costs.

The fruit SSC is influenced by the genome as well as by the environmental factors. In recent approaches (Hutton *et al.* 2007), the effect of temporal water supply on the fruit quality was studied. The spatially and temporal resolved variance of fruit quality can be approached again by means of non-destructive fruit monitoring. In viticulture, site-specific data recording already has a longer history (Johnson *et al.* 2001; Hall *et al.* 2003; Lamb *et al.* 2004; Bramley and Hamilton 2007; dos Santos *et al.* 2007; Reynolds *et al.* 2007). A citrus production site with low planting distance and pruning measures for small and loose, spindle-like canopies compared to a traditional planting system providing higher plant distances and high density, bigger canopies, the standard deviation of fruits from inner canopy and exposed sections in the canopy was randomly distributed in the sites. The standard deviation was generally higher in the traditional system with dense canopies (**Fig. 6**). Such findings point to the potential value of monitoring fruits by means of *in-situ* analysis regarding the spatio-temporal variability in the fruit maturity stage in the production. By means of the data, the site-specific harvest measures can be optimized with respect to heterogeneity of the crop and efficient labour usage. Performing spatially-resolved, cost-effective production measures (Sevier and Lee 2004; Bullock and Lowenberg-de Boer 2007; Reidsma *et al.* 2007) are gaining more support by the development of autonomous vehicles (e.g. Subramanian *et al.* 2006).

CONCLUDING REMARKS

In-situ analyses for monitoring the physiological plant response may become an important tool to deal with necessary changes in crop production in the future. Beside fine-tuning the processes based on the physiological plant response and making the processes more efficient, present research regarding to the impact of climate change shows a new application field of *in-situ* analyses. Data on the crop are presently hardly available with respect to spatial and temporal changes of the crop development.

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