

# The Potential of a Chlorophyll Content SPAD Meter to Quantify Nutrient Stress in Foliar Tissue of Sycamore (*Acer pseudoplatanus*), English Oak (*Quercus robur*), and European Beech (*Fagus sylvatica*)

Glynn C. Percival, Ian P. Keary, and Kelly Noviss

**Abstract.** The chlorophyll content (or SPAD meter) is a simple, portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves. Compared with the traditional destructive methods of chlorophyll extraction, the use of this equipment saves time, space, and resources. The objective of this study was to establish a correlation between the leaf photosynthetic pigment content (chlorophylls, carotenoids) extracted in aqueous acetone, total leaf nitrogen (N) content, and chlorophyll fluorescence Fv/Fm values with the SPAD-502 readings in sycamore (*Acer pseudoplatanus*), beech (*Fagus sylvatica*), and English oak (*Quercus robur*) leaves displaying visual symptoms of N deficiency. In addition, this study aimed to determine a critical foliar N content below which a reduction in photosynthetic efficiency occurs. Irrespective of species, high correlations were recorded between SPAD readings, total leaf chlorophyll and carotenoid content, foliar N content, and leaf photosynthetic efficiency as measured by chlorophyll fluorescence Fv/Fm values; however, a poor correlation between SPAD values and total chlorophyll:carotenoid ratios was obtained. In the case of *Acer pseudoplatanus*, *Fagus sylvatica*, and *Quercus robur*, SPAD readings lower than 25 indicated impairment of leaf photosynthetic process that in turn were correlated with a foliar N content less than 1.5%, a value associated with a critical N deficiency. Results of this study indicate that the chlorophyll content SPAD-502 m potentially offers a useful nondestructive, handheld system to aid in the evaluation of tree health. However, users should be aware of the limitations of this system. Consistency in sample collection and seasonal timing may necessitate species and cultivar calibration equations to correlate SPAD values with reductions in tree vitality.

**Key Words.** Carotenoids; chlorophyll fluorescence; chlorophylls; light transmittance; nitrogen fertilization; stress detection; tree evaluation.

Urban environments present an array of environmental factors hostile to the biology of trees (soil deoxygenation, compaction, aerial pollution, and deicing salts). These stresses limit the amount of carbohydrates available for growth and reduce nutrient uptake resulting in leaf chlorosis and necrosis (Jimenez et al. 1997; Mohammed et al. 1997; Maki and Colombo 2001). These symptoms become manifest as leaf yellowing that are visible indicators arborists interpret to assess tree vitality (Percival 2004). Visual observations can be very subjective because they are based on human knowledge and interpretation that can significantly differ between individuals (Percival 2004). Consequently, health evaluations can be markedly different between assessors. Field diagnostic tools are now required to objectively evaluate stress disorders in trees as a basis for management decisions on cultural practice and for proactive monitoring in urban treescapes (Loh et al. 2002). Such diagnostic instruments and methodologies should ideally detect stress before the visible symptoms of plant deterioration become manifest and therefore make possible effective remedial intervention (Percival and Fraser 2001).

Both biotic and abiotic stress factors affect the content and efficiency of leaf photosynthetic pigments and/or their reciprocal ratio (Bacci et al. 1998). For example, the assessment of leaf photosynthetic pigments is an important indicator of senescence because breakdown of leaf chlorophyll is associated with environmental stress (Brown et al. 1991). Likewise, the variation in

total chlorophyll/carotenoids ratio has been used as a useful indicator of stress in plants because a rapid increase in total leaf carotenoid content is a recognized plant stress response (Hendry and Price 1993). Exact knowledge of foliar chlorophyll concentrations, i.e., “greenness,” consequently may provide a robust and accurate estimation of tree vitality. Traditional methods of extracting chlorophylls from leaves using chemical solvents such as acetone, dimethylsulfoxide, and methanol require laboratory conditions, are time-consuming, and involve destructive leaf analysis (Lichtenthaler and Wellburn 1983).

The chlorophyll content meter (or SPAD meter) is a commercially available portable piece of equipment that is used to measure greenness based on optical responses when a leaf is exposed to light that in turn is used to estimate foliar chlorophyll concentrations (Kariya et al. 1982). The meter makes instantaneous and nondestructive readings on a plant based on the quantification of light intensity (peak wavelength: approximately 650 nm: red light-emitting diode [LED]) absorbed by the tissue sample. A second peak (peak wavelength: approximately 940 nm: infrared LED) is emitted simultaneous with red LED to compensate for thickness of the leaf (Hoel 1998).

Past research has shown a close link between leaf chlorophyll concentration and leaf nitrogen (N) content in agricultural crops such as rice, maize, and wheat because the majority of leaf N is contained within the chlorophyll molecules (Peterson et al. 1993). Consequently, chlorophyll content meters are widely used

to detect N deficiencies and subsequently improve N management within the agricultural industry (Peterson et al. 1993; Smeal and Zhang 1994; Balasubramanian et al. 2000). The use of a chlorophyll SPAD meter for monitoring the N status of woody plants under field conditions has received limited attention (Loh et al. 2002).

Several studies have shown that carotenoids are vitally important in protecting the leaf photosynthetic apparatus, especially photosystems I and II, against photoinhibition under prolonged environmental stress by interconversions among the xanthophyll molecules (Hall and Rao 1999; Ort 2001). In the xanthophyll cycle, violaxanthin goes through de-epoxidation to give rise to antheroxanthin and finally zeaxanthin that, together with a low pH within the photosynthetic membrane, facilitate the harmless dissipation of excess excitation energy directly within the light-harvesting chlorophyll antennae (Havaux 1988; Ramalho et al. 2000). Therefore, an indirect, nondestructive quantification of total leaf carotenoids will prove important for stress-related studies (Torres Netto et al. 2005).

Chlorophyll fluorescence, an indication of the fate of excitation energy in the leaf photosynthetic apparatus, has been used to provide a rapid and nondestructive diagnostic system of detecting and quantifying physiologic injury in tree leaves and needles (photosynthetic organs) under low temperatures, salinity, and water stress conditions (Sestak and Stiffel 1997; Percival and Fraser 2001; Percival and Sheriffs 2002; Percival and Henderson 2003). Little information associating fluorescence values as measures of damage to the leaf photosynthetic system and readings with a chlorophyll SPAD meter exist (Torres Netto et al. 2005). Association between SPAD and fluorescence can be important to determine SPAD values associated with reductions in leaf photosynthetic properties induced by nutrient deficiency-related disorders.

## MATERIALS AND METHODS

### Plant Material and Growth Conditions

Mature, fully expanded leaves near the top of the canopy (generally about the fourth leaf from the apex) of five sycamore (*Acer pseudoplatanus*), beech (*Fagus sylvatica*), and English oak (*Quercus robur*) displaying a range of visually different leaf colors indicating a range of chlorophyll concentrations were selected. In all cases, leaf tissue was collected and sampled in late June 3 months after leaf flush, a time when leaves show maximum photosynthetic performance (Kitao et al. 1998). All trees were located in commercial plantings at the University of Reading campus (51°43' N, -1°08' W). The sampled leaves were transported in insulated boxes sheltered from light and all material prepared within 2 hr of collection.

### SPAD Readings

The mean of three readings from a portable Minolta chlorophyll meter SPAD-502 (Spectrum Technologies, Inc., Plainfield, IL, U.S.) was obtained for each leaf disc from individual leaves (10 leaves per tree) and pooled to obtain one SPAD measurement per disc. The leaf disc used to obtain a SPAD value provided sufficient tissue for total chlorophyll and carotenoid content as well as chlorophyll fluorescence Fv/Fm quantification. In the case of total foliar N content, however, six leaf discs were required. Consequently, SPAD values for all six discs (60 leaves per tree) were pooled for statistical purposes when comparing SPAD measurements versus total leaf N content. SPAD values were

measured at the midpoint of the leaf next to the main leaf vein. This position was selected because examination of the relationship between SPAD readings taken at different positions on a leaf concluded this position most closely correlated with total leaf N and protein content as well as plant yield (Hoel 1998). This position was also most convenient from a practical point of view (Hoel 1998).

### Total Leaf Chlorophyll and Carotenoid Content

Quantification was obtained by measurement of absorbance at 663, 645, and 480 nm in a spectrophotometer (PU8800 Pye Unicam, Portsmouth, U.K.) after extraction with 80% v/v aqueous acetone. Chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations were determined according to the equation of Lichtenthaler and Wellburn (1983).

### Nitrogen Content Assessment

Six leaf discs per N analysis were thoroughly washed and then dried in a convection oven at 85°C (185°F) for 48 hr before grinding through a 0.5 mm (0.02 in) cyclone mill (Retsch, Middlesborough, U.K.). Each six discs were placed in specific groups according to the SPAD-502 reading ranges (0–10, 11–20, 21–30, 31–40, 41–50, 51–60). Samples were placed into 150 mL (4.5 fl oz) volumetric flasks and digested in 20 mL (0.6 fl oz) of 7:1 nitric/perchloric acid. After cooling, the solutions were brought to volume with deionized water and analyzed by inductively coupled plasma-emission spectroscopy elemental analysis. Nutrient values were expressed as percent total leaf dry weight.

### Chlorophyll Fluorescence Fv/Fm Measurements

Chlorophyll fluorescence was determined on leaf discs using a dark-acclimated Handy Plant Efficiency Analyzer chlorophyll fluorometer (Hansatech Instruments, King's Lynn, Norfolk, U.K.). The initial fluorescence ( $F_0$ ) and maximum fluorescence ( $F_m$ ) were analyzed and quantum efficiency of open photosystem II centers—quantum yield ( $F_v/F_m$ ) calculated. The leaf discs were previously adapted to the dark for 30 min so that all the centers of photosystem II (PSII) were at an open stage (all the primary acceptors oxidized) and energy dissipation through heat was minimal. The  $F_0$  was obtained with low-intensity light (less than  $0.1 \mu\text{mol}/\text{m}^2/\text{s}^{-1}$ ) not to induce any effect in the fluorescence variable. The  $F_m$  was obtained by a continuous light excitation (at  $2500 \mu\text{mol}/\text{m}^2/\text{s}^{-1}$ ) provided by an array of six LEDs focused on the leaf surface to provide homogeneous irradiation over a 4 mm (0.16 in) diameter leaf surface. The fluorescence variable ( $F_v$ ) was calculated from the difference between  $F_m$  and  $F_0$ . The  $F_v$  and  $F_m$  values were used to obtain the  $F_v/F_m$  ratio.

### Statistical Analysis

Correlation equations and coefficients of multiple determinations ( $r^2$ ) were calculated using the curve-fitting feature of Genstat V using quadratic polynomial, logarithmic, exponential growth or decay or simple linear models as appropriate according to which model gave the highest percentage variation, i.e., goodness of fit, accounted for.

## RESULTS AND DISCUSSION

### SPAD versus Total Chlorophyll

Figure 1 shows the relationships between SPAD readings and total leaf chlorophyll concentrations in three tree species. A polynomial quadratic mathematical model best fit the relation-

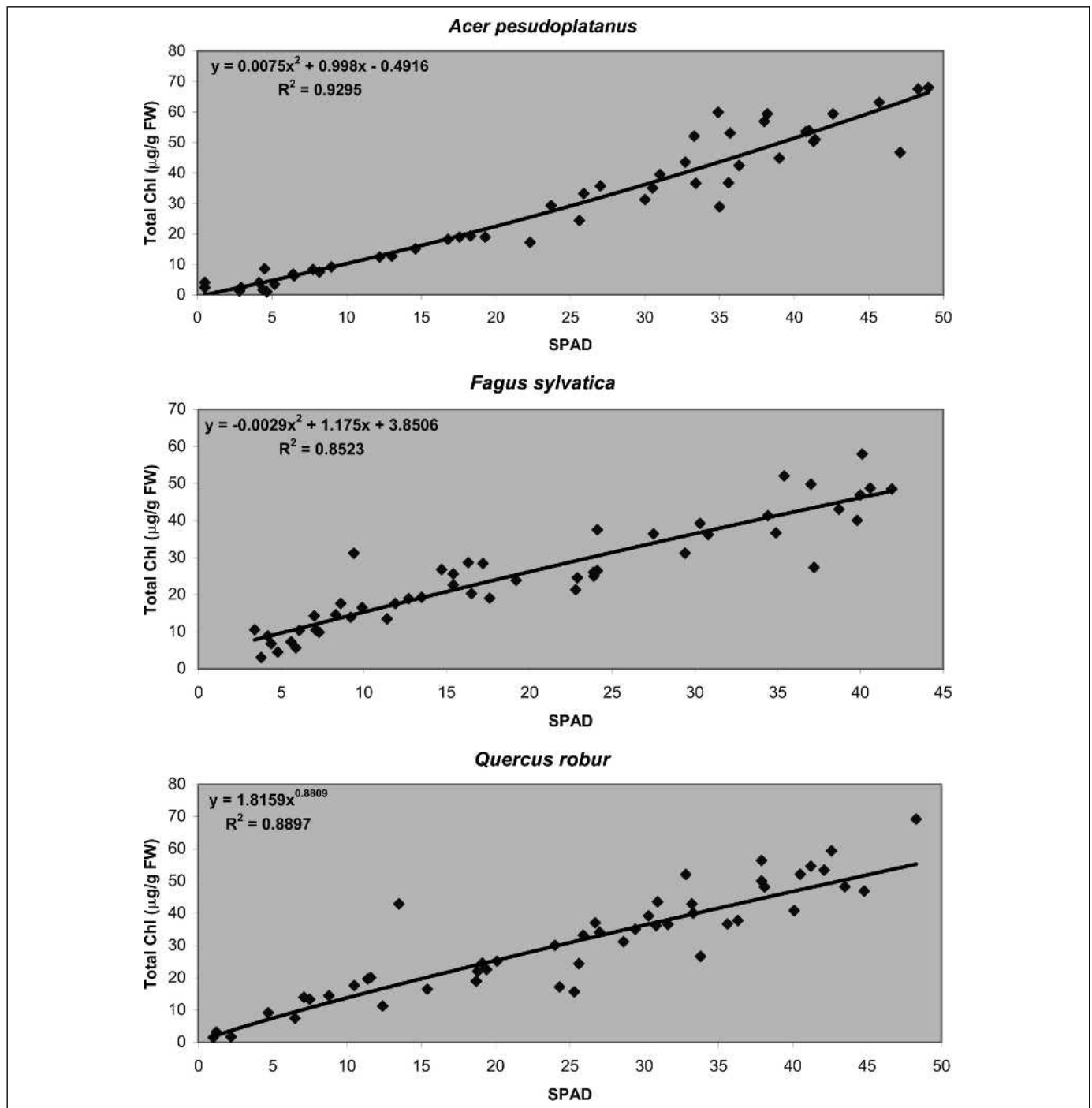


Figure 1. Calibration curves for a SPAD-502 chlorophyll content meter versus total leaf chlorophyll (Chl) concentration in three tree species. Leaves were collected from mature trees in late June 3 months after leaf flush. FW = fresh weight. For regression equations and  $R^2$ , see "Materials and Methods."

ship between these parameters with  $R^2$  values ranging between 0.83 and 0.93. Similar relationships among total leaf chlorophyll concentration and SPAD readings have been established with other plant species (Yadava 1986; Marquard and Tipton 1987; Schaper and Chacko 1991). Traditional methods of extracting chlorophylls from leaves using chemical solvents require laboratory conditions and are time-consuming, labor-intensive, and expensive. Results here, and those elsewhere, indicate a SPAD

meter can be used to measure greenness based on optical responses when a leaf is exposed to light that in turn is used to accurately estimate foliar chlorophyll concentrations. The importance of this relationship may have many applications to arborists. Because leaf chlorophyll concentrations change in response to external factors such as light and after various pruning regimes, building removal, or constructions activities, quantifying chlorophyll concentrations may provide important informa-

tion about tree growth and physiologic plasticity in response to changing environments (Larcher 1995; Richardson et al. 2002). The amount of solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments. Low concentrations of chlorophyll can therefore directly limit photosynthetic potential and hence primary production (Filella et al. 1995). A SPAD meter is ideally suited for the arborist requiring minimal training in its use and no detailed scientific background knowledge. Analysis is rapid (1 to 2 sec per reading) allowing for many trees to be evaluated in a single day. Importantly, measurements are nondestructive and noninvasive allowing for periodic repetitive sampling (Loh et al. 2002; Richardson et al. 2002)

### SPAD versus Total Carotenoids

Quadratic polynomial (*Acer pseudoplatanus*) and logarithmic regression (*Fagus sylvatica*, *Quercus robur*) models were adequate in explaining the relationships between SPAD and total leaf carotenoids with  $R^2$  of 0.85, 0.84, and 0.82, respectively (Figure 2). Results show that an indirect carotenoid quantification can be obtained for SPAD values up to 50 using the SPAD-502 despite the 650 nm quantifying system that is the wavelength relevant to chlorophyll absorption (Torres Netto et al. 2005). These inferences can be obtained as a result of the direct relationship between the total chlorophyll and carotenoid concentration within leaves. In higher plants, carotenoids generally consist of 7% to 9% of total leaf photosynthetic pigments, consistent with values obtained in this study for all three tree species (Hall and Rao 1999; Lawlor 2001). The importance of a nondestructive means of quantifying carotenoid concentrations lies in the fact that an initial plant stress response is stomatal closure to conserve transpirational water loss. Such a response can be highly detrimental to the leaf photosynthetic system as a result of the prevention of light energy conversion into photochemical energy caused by low  $CO_2$  concentrations within the leaf tissue in turn resulting in the production of high-energy reactive oxygen species (ROS) such as superoxide and singlet oxygen (Lawlor 2001). Buildup of ROS results in oxidization damage to leaf membranes, i.e., chlorophyll bleaching and cellular membrane destruction. To minimize the effects of oxidative stress, plants have evolved an antioxidant system consisting of carotenoids that function as protective photooxidative pigments responsible for the quenching of these ROS (Kraus and Fletcher 1994). Because an increase in total leaf carotenoid content is a widely recognized plant stress response (Peñuelas and Filella 1998), quantification of total leaf content can provide indicators of plant responsiveness to stresses frequently encountered in urban and landscape environments (Strauss-Debenedetti and Bazzaz 1991; Hendry and Price 1993; Vieira 1996).

### SPAD versus Total Chlorophyll:Carotenoid Ratio

A poor relationship between the total leaf chlorophyll:carotenoid ratio was shown for all three species. Goodness of fit  $R^2$  values of 0.49 (*Acer pseudoplatanus*), 0.54 (*Fagus sylvatica*), and 0.13 (*Quercus robur*) were recorded using quadratic polynomial regression models (Figure 3). Contrary to this, the ratio between chlorophyll and carotenoids has been shown to be a sensitive marker distinguishing natural senescence, senescence resulting from environmental stresses (Buckland et al. 1991), drought, and photooxidative damage in plants (Seel et al. 1992; Hendry and Price 1993). The poor relationship recorded in this study may

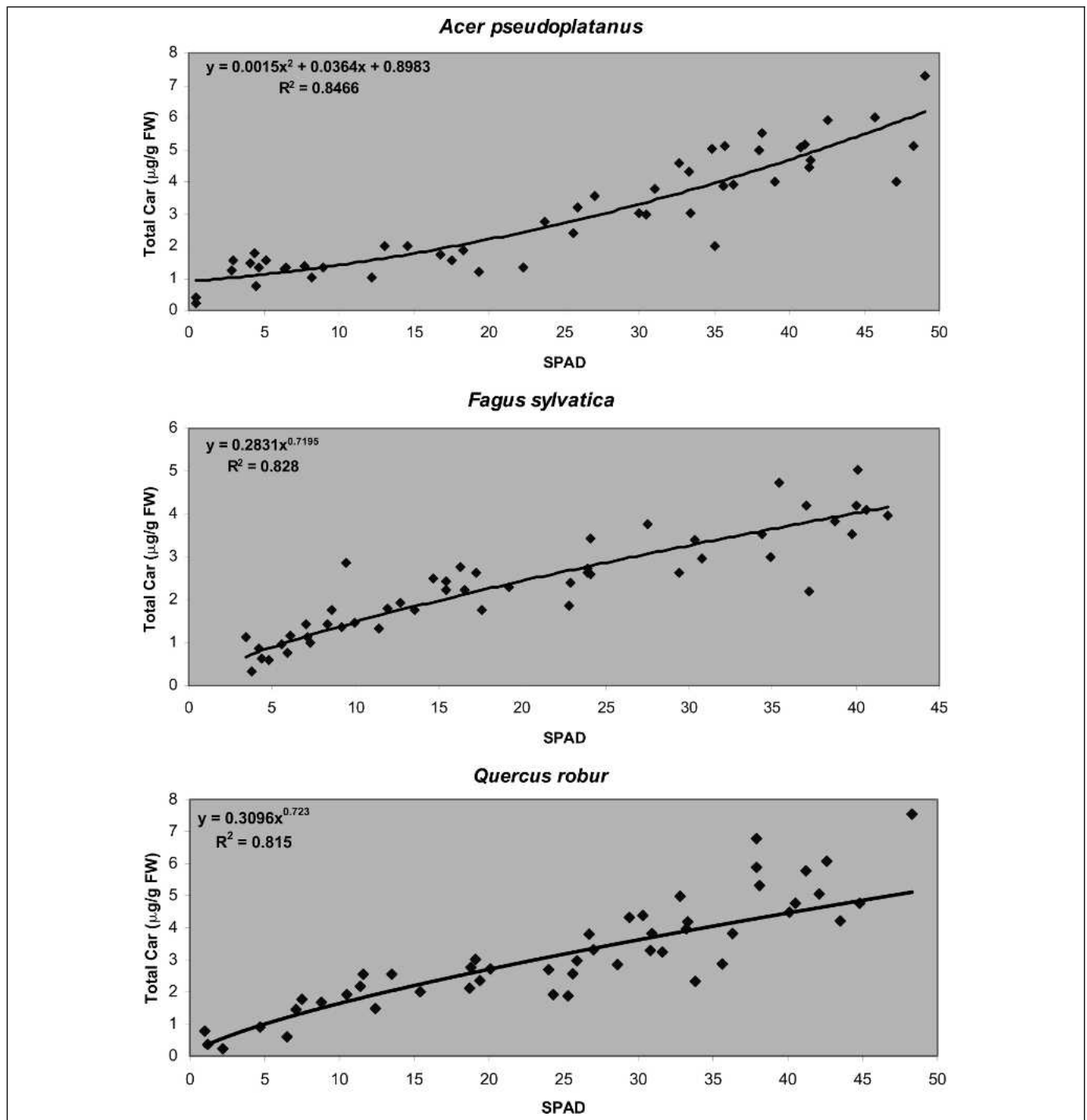
relate to the type of stress imposed. An adequate N supply is essential for the formation of chloroplast and carotenoid structure (Peoples et al. 1980). When N availability is low, both the leaf chlorophyll and carotenoid content are reduced (Doncheva et al. 2001). However, analysis of the ultrastructure of leaves indicated a more marked influence of N deficiency on leaf chlorophyll content compared with carotenoid content, which may account for this poor correlation (Peñuelas and Filella 1998; Doncheva et al. 2001). Results of this investigation therefore indicate that leaf chlorophyll:carotenoid ratios do not provide as robust a system of identifying stress disorders in trees caused by N deficiency compared with other plant vitality systems such as chlorophyll fluorescence Fv/Fm values used in this investigation.

### SPAD versus Chlorophyll Fluorescence (Fv/Fm)

Leaf chlorophyll content is often well correlated with leaf photosynthetic rates (Evans 1983; Seeman et al. 1987). Correlations between SPAD values and Fv/Fm values as measures of photosystem II efficiency are limited. According to the quadratic fitted model, the maximum quantum efficiency of the photosystem II, indicated by the  $F_v/F_m$  ratio, started to fall at around a SPAD value of 25 (Figure 4) irrespective of species. Exceedingly high goodness of fit values ( $R^2$  greater than 0.94) were associated with these models. The Fv/Fm ratio is positively correlated to the PSII quantum yield and an indirect measurement of plant physiologic status (Kitajima and Butler 1975; Maxwell and Johnson 2001) for which values of  $0.8 \pm 0.05$  correspond to highly efficient use of the excitation energy in photochemical processes (Björkman and Demmig 1987; Mohammed et al. 1995; Percival 2005). Consequently, results of this investigation indicate that SPAD readings around 25 appear to be the start of PSII impairment caused by N deficiency in the species used in this study. Past research by Percival (2004) and Maki and Colombo (2001) indicated Fv/Fm values of 0.6 below which trees were affected in terms of reduced survival, height growth, and foliar necrosis. Fv/Fm ratios of 0.6 were associated with SPAD values between 15 and 20 in this study. However, this study was conducted on three tree species at one stage during the growing season. Consequently, although preliminary results are promising, further work is needed to assess the applicability of SPAD values versus reductions in photosynthetic efficiency as measured by chlorophyll fluorescence for other ornamental tree species. An example of the dangers of extrapolating SPAD values from one plant species to another can be gauged by reference to work by Torres Netto et al. (2005), who concluded SPAD values less than 40 were correlated with reductions in photosynthetic efficiency as measured by Fv/Fm values, not 25 as reported in this study. Such a response indicates individual regression models need to be developed for differing species and cultivar.

### SPAD versus Leaf Nitrogen Content

Nitrogen is one of the most important factors in plant growth physiology. It is related to leaf photosynthetic rate (Evans 1989), dark respiration (Anten et al. 2000), quantum yield (Hirose and Werger 1987), leaf extension (Gastal et al. 1992), mesophyll size (Körner 1989), leaf aging (Escudero and Mediavilla 2003), leaf lifespan (Hikosaka and Hirose 2000), and leaf chlorophyll concentration (Pons et al. 1994). Optimal correlations between total leaf N concentrations and SPAD values were obtained using quadratic (*Acer pseudoplatanus*, *Quercus robur*) and linear (*Fagus sylvatica*) regression models with higher SPAD values re-



**Figure 2.** Calibration curves for a SPAD-502 chlorophyll content meter versus total leaf carotenoid (Car) concentration in three tree species. Leaves were collected from mature trees in late June 3 months after leaf flush. FW = fresh weight. For regression equations and  $R^2$ , see "Materials and Methods."

flecting higher internal foliar N content (Figure 5). In rice leaves, Takebe and Yoneyama (1989) showed a strong linear relationship between SPAD readings and weight-based leaf N concentration that varied with crop growth stage and variety, mainly as a result of leaf thickness or specific leaf weight (Peng et al. 1995). The confounding effect of leaf thickness can be eliminated if foliar N concentration is expressed on a leaf area basis (Balasubramanian et al. 2000); however, when SPAD values are

adjusted for this, the chlorophyll meter estimation is no longer as quick, simple, or nondestructive as the unadjusted SPAD value (Hoel 1998). Likewise, the SPAD-502 m has been shown to be accurate in predicting chlorophyll and N levels in maize (*Zea mays*) (Wood et al. 1992) and wheat (*Triticum aestivum*) (Follett et al. 1992). Results of this investigation demonstrated a high model fit between SPAD and total N content in the three test trees under field conditions. Plant N status is of interest to land-

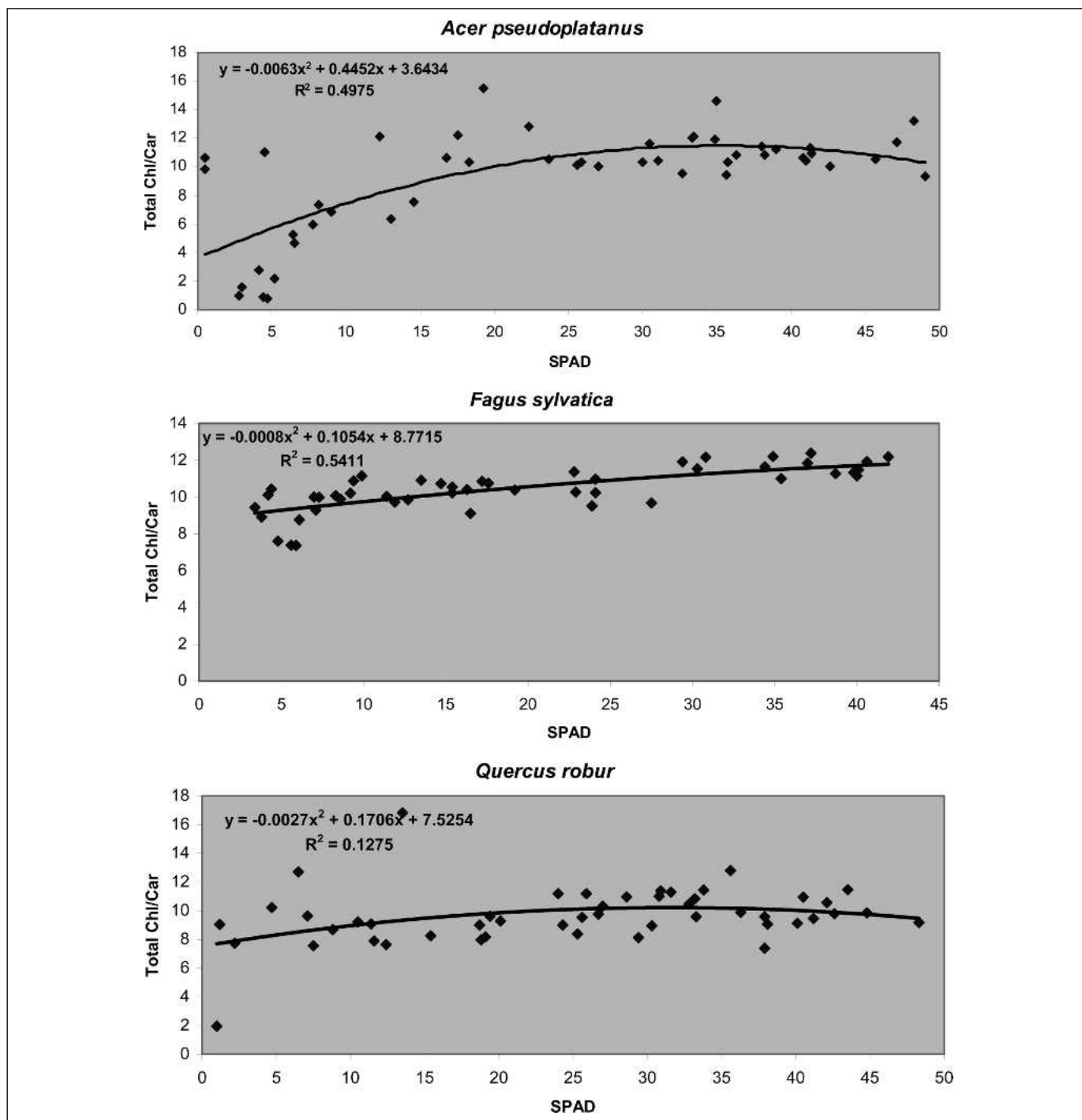
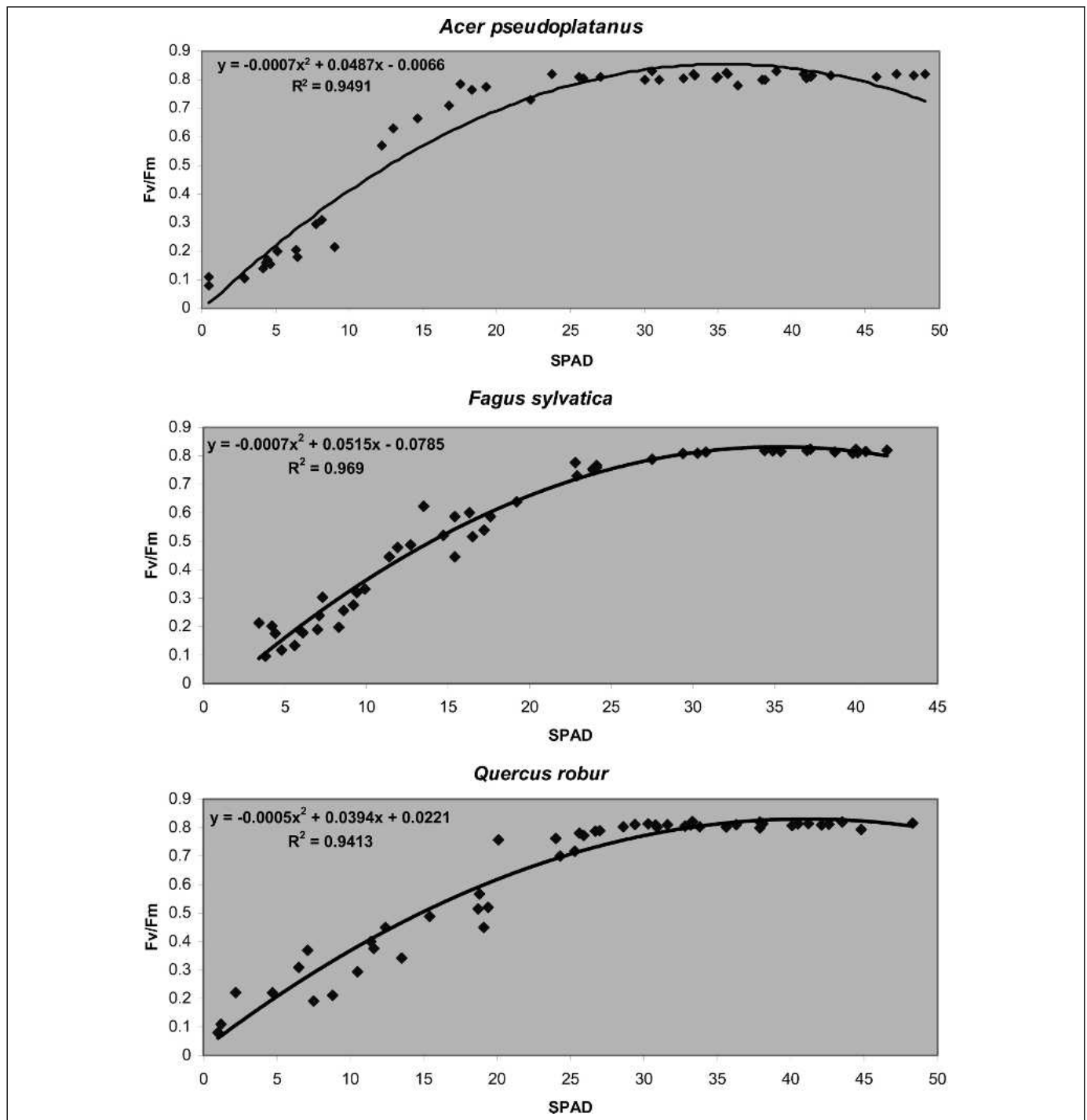


Figure 3. Calibration curves for a SPAD-502 chlorophyll content meter versus total leaf chlorophyll (Chl):carotenoid (Car) concentration in three tree species. Leaves were collected from mature trees in late June 3 months after leaf flush. FW = fresh weight. For regression equations and  $R^2$ , see "Materials and Methods."

scape managers, because foliar N content influences tree aesthetics, vigor, pests and disease susceptibility as well as ability to tolerate environmental stress. By using this tool, it may be possible to synchronize fertilizer N application with actual tree demand. Data from Cresswell and Weir (1997) indicate the percentage leaf N associated with woody plant health ranges between 1.7% and 2.5% with values less than 1.7% generally associated with a low foliar N content. Results of this study

indicate the SPAD threshold or critical value indicating low foliar N (less than 1.7%) in the three trees used for test purposes ranges between 22 and 25. Consequently, results of this investigation indicate a SPAD value less than 22 as a level when N fertilization should start in these three tree species to prevent N-related deficiency problems. In the case of rice genotypes, a SPAD threshold value of 35 is generally recognized as a critical value (Peng et al. 1996). Whenever SPAD readings fall below



**Figure 4.** Calibration curves for a SPAD-502 chlorophyll content meter versus leaf chlorophyll fluorescence (Fv/Fm) as a measure of photosynthetic efficiency in three tree species. Leaves were collected from mature trees in late June 3 months after leaf flush. For regression equations and  $R^2$ , see "Materials and Methods."

the 35 critical value, the rice crop suffers from N deficiency, and yields will decline if N fertilizer is not applied. Differing SPAD values associated with N deficiency in rice and trees may relate to the fact that rice genotypes have over the past 100 years been bred and selected for high vigor and yields. To achieve these aims, high inputs of N fertilizers are required and as a result of these breeding programs, most rice genotypes are derived from a very narrow genetic base. Such criteria are rarely considered

with amenity trees where aesthetics, shape, form, and size are more important criteria for selection (Percival and Hitchmough 1995). Consequently, amenity trees are not regarded as high N demanders in comparison to agricultural crops and a single tree genus can consist of many species with differing growth forms and leaf, flower, bark, and berry characteristics, i.e., differing tree genera contains a very broad genetic base for selection. In addition, Loh et al. (2002) warns of the difficulties in extending

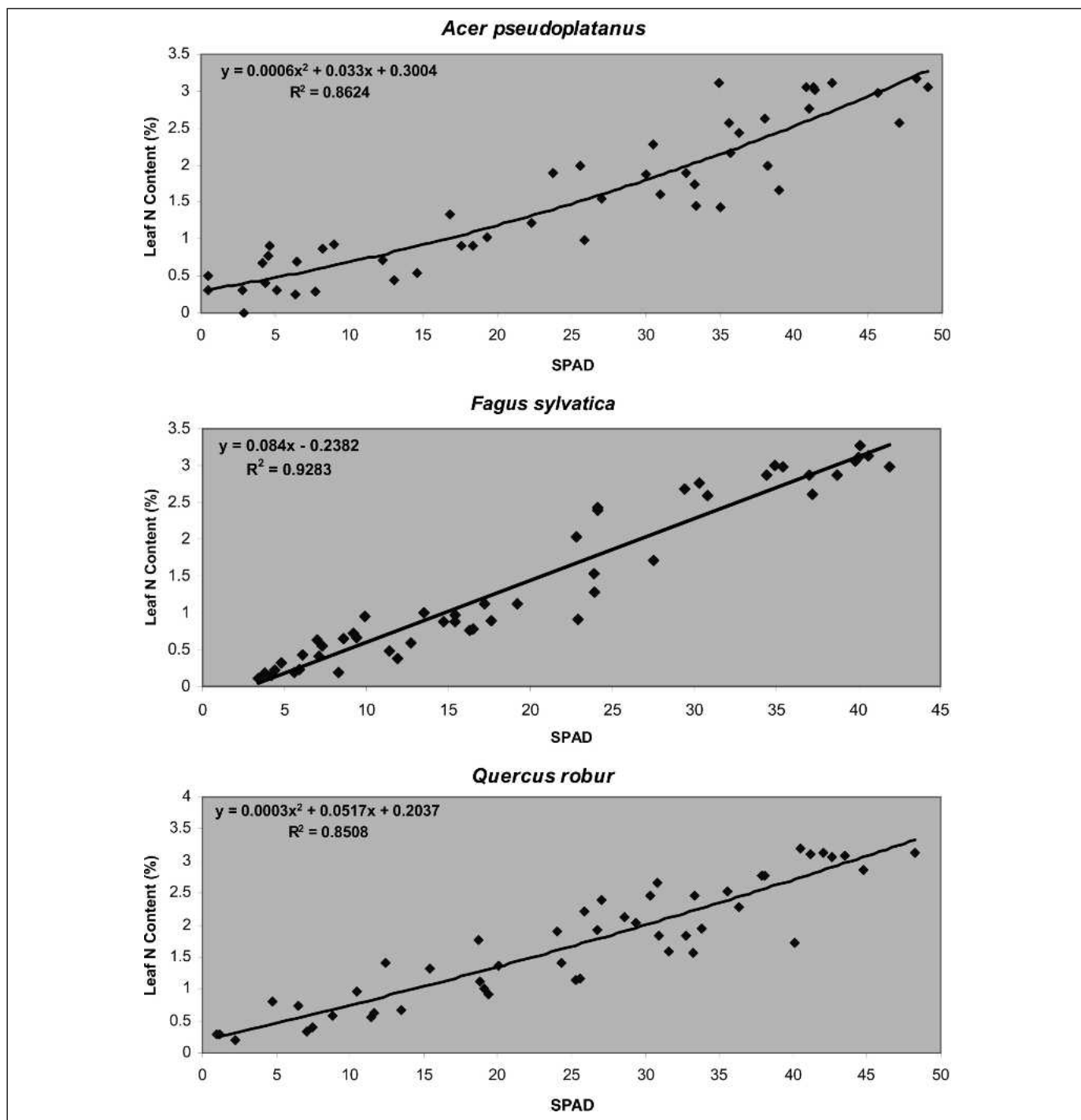


Figure 5. Calibration curves for a SPAD-502 chlorophyll content meter versus total leaf nitrogen (N) concentration in three tree species. Leaves were collected from mature trees in late June 3 months after leaf flush. FW = fresh weight. For regression equations and  $R^2$ , see "Materials and Methods."

SPAD technology to detect nutrient levels, particularly N content in perennial plants. Issues of sampling protocol such as leaf physiologic age, position, sampling time, interactions with other mineral content, and complex source–sink relationships associated with perennial plants are likely sources of variability. These are all valid points supported by research by Peterson et al. (1993), Takebe and Yoneyama (1989), Turner and Jund (1994), and Peng et al. (1993). Consequently, extrapolating the SPAD

value recorded in this investigation to other woody plants is not recommended.

#### Chlorophyll Fluorescence (Fv/Fm) versus Leaf Nitrogen Content

Reductions in photosynthetic efficiency caused by N deficiency have been reported elsewhere, because the amount of light absorbed by a leaf is largely a function of leaf chlorophyll con-



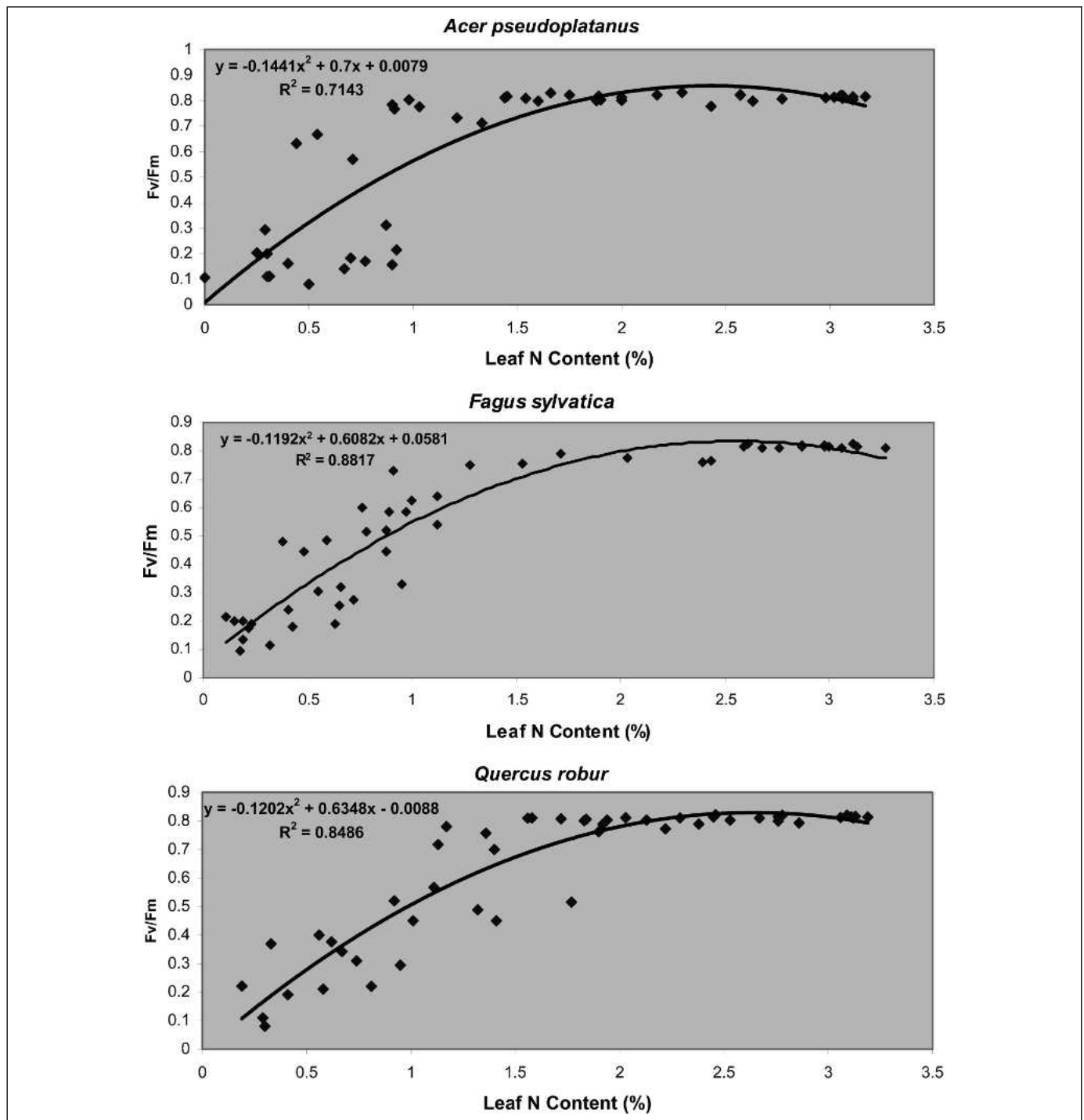


Figure 6. Calibration curves of total leaf nitrogen (N) concentration versus leaf chlorophyll fluorescence (Fv/Fm) as a measure of photosynthetic efficiency in three tree species. Leaves were collected from mature trees in late June 3 months after leaf flush. For regression equations and  $R^2$ , see "Materials and Methods."

centration (Evans 1989; Filella et al. 1995). Lower photosynthetic efficiency caused by lower foliar N content could be anticipated because the majority of leaf N is contained within the chlorophyll molecules (Peterson et al. 1993) that in turn act as the major cell of photosynthetic activity within higher plants (Lawlor 2001). Low chlorophyll concentrations limit photosynthesis and therefore tree growth. However, the minimal leaf N

content required for functional photosynthetic efficiency in urban trees remains largely unknown. According to Cresswell and Weir (1997), the percentage leaf N associated with a standard required for tree health ranges between 1.7% and 2.5% to include species from the *Betula*, *Fagus*, *Buxus*, *Abies*, *Fraxinus*, *Ilex*, *Juniperus*, *Larix*, *Pinus*, and *Picea* genus. Values less than 1.7% are generally associated with a low foliar N content and

values less than 1.5 associated with a critical deficiency; however, variation between species does exist. In agreement with the standards stipulated by Cresswell and Weir (1997), quadratic regression models ranging between 0.71 and 0.88  $R^2$  values in all three tree species (Figure 6) indicate a foliar N content less than 1.5 is associated with reductions in Fv/Fm values of 0.8 (values associated with full photosynthetic functioning) (Björkman and Demmig 1987; Mohammed et al. 1995; Percival 2005). Foliar N content between 0.8% and 1.2% corresponds to Fv/Fm values of 0.6 which in turn relate to reduced survival and growth (Maki and Colombo 2001; Percival 2004). Results presented here are one of the first to quantify actual foliar N content with impairment of the leaf photosynthetic system and potential influence on future growth and survival.

In conclusion, results of this study indicate that the chlorophyll content SPAD meter potentially offers a useful nondestructive, handheld system to aid in the evaluation of tree health. High correlations were obtained among SPAD readings, total leaf chlorophyll and carotenoid content, foliar N content, and leaf photosynthetic efficiency as measured by chlorophyll fluorescence Fv/Fm values. A lower correlation between SPAD values and total chlorophyll/carotenoid content were obtained. In the case of *Acer pseudoplatanus*, *Fagus sylvatica*, and *Quercus robur*, SPAD readings lower than 25 indicated impairment of the leaf photosynthetic process. However, critical chlorophyll meter values indicating reductions in tree vitality may vary among species and among cultivars within the same species (Hoel 1998). Likewise, the chlorophyll content of a leaf varies with age. Consequently, consistency in sample collection and seasonal timing may be necessary to develop species and cultivar calibration to correlate SPAD values with reductions in tree vitality (Hoel 1998).

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Glynn C. Percival (corresponding author)  
 Plant Physiologist/Technical Support Specialist  
 R.A. Bartlett Tree Research Laboratory, Europe  
 The University of Reading  
 2 Early Gate, Whiteknights  
 Reading, RG6 6AU, U.K.  
 gpercival@bartlettuk.com

Ian P. Leary  
 Research Graduate  
 R.A. Bartlett Tree Research Laboratory, Europe  
 The University of Reading  
 2 Early Gate, Whiteknights  
 Reading, RG6 6AU, U.K.

Kelly Noviss  
 Research Assistant  
 R.A. Bartlett Tree Research Laboratory, Europe  
 The University of Reading  
 2 Early Gate, Whiteknights  
 Reading, RG6 6AU, U.K.

**Résumé.** Le mesureur de contenu en chlorophylle SPAD-502 est un outil simple et portatif de diagnostic qui mesure le contenu relatif en chlorophylle des feuilles. L'objectif de cette étude était d'établir une corrélation entre le contenu en pigment foliaire photosynthétique (chlorophylle, caroténoïdes) extrait à partir de l'acétone aqueuse, le contenu en azote foliaire et les valeurs de fluorescence Fv/Fm à partir des lectures du SPAD-502 sur des feuilles d'érable sycamore (*Acer pseudoplatanus*), de hêtre européen (*Fagus sylvatica*) et de chêne anglais (*Quercus robur*) qui présentaient des symptômes visuels de déficience en azote. Peu importe l'espèce, des corrélations élevées ont été enregistrées entre les lectures du SPAD-502 et le contenu total en chlorophylle foliaire et en caroténoïdes, le contenu foliaire en azote et les valeurs Fv/Fm de fluorescence de la chlorophylle; cependant, une corrélation faible a été obtenue entre les valeurs du SPAD et les ratios totaux de chlorophylle: caroténoïdes. Pour les trois espèces, des lectures de SPAD inférieures à 25 indiquaient une détérioration du processus photosynthétique foliaire qui était en retour corrélé avec un contenu en azote foliaire <1,5%. Les résultats de cette étude indiquent que la mesure du contenu en chlorophylle à partir du SPAD-502 offrent potentiellement un système très utile, non destructif et à portée de main pour aider à évaluer la santé d'un arbre. Néanmoins, les utilisateurs devraient être prévenus des limites de ce système. L'uniformité dans la collecte des échantillons ainsi que la période où ils sont recueillis pourraient nécessiter l'utilisation d'équations de calibration en fonction des espèces et des cultivars afin de corréler les valeurs du SPAD avec les diminutions de vitalité de l'arbre.

**Zusammenfassung.** Das Messgerät für Chlorophyllgehalt SPAD-502 ist ein simples, tragbares Diagnose-Gerät, welches den relativen Chlorophyllgehalt von Blättern misst. Das Ziel dieser Studie war, eine Korrelation zwischen dem Blattgehalt an photosynthetisch aktiven Pigmenten (Chlorophyll, Carotin), extrahiert in Acetonlösung, dem totalen N-Gehalt der Blätter und den Chlorophyll-Fluoreszenz Fv/Fm-Werten mit den Messungen des SPAD-502 bei Bergahorn, Buche und Roteichenblättern, die sichtbare Symptome von N-Mangel zeigten. Unabhängig von der Species wurden hohe Korrelationen zwischen den SPAD-Messungen, dem totalen Chlorophyll- und Carotengehalt, Blattstickst-

offgehalt und den Chlorophyll-Fluoreszenz Fv/Fm-Werten, aber es bestand eine geringe Korrelation zwischen den SPAD-Werten und dem totalen Chlorophyll-Carotin-Verhältnis. Bei allen drei Arten, zeigten die SPAD-Werte unter 25 eine Verringerung des Blattphotosyntheseprozesses, welcher im Gegenzug mit einem Blattstickstoffgehalt <1,5 korrelierte. Die Ergebnisse dieser Studie zeigen, dass der SPAD-502-Messgerät potentiell ein nützliches, zerstörungsfreies, tragbares System zur Bewertung von Baumgesundheit darstellt. Dennoch sollten sich die Anwender der Grenzen dieses Systems bewusst sein. Die Beschaffenheit der Proben und der saisonale Zeitraum erfordern eine Kalibrierung der Arten und Kultivare, um die SPAD-Werte mit den Vitalitätsverlusten der Bäume zu korrelieren.

**Resumen.** El medidor de contenido de clorofila SPAD-502 es una herramienta simple, portátil, que mide el contenido relativo de clorofila de las hojas. El objetivo de este estudio fue establecer una correlación entre el contenido de pigmento fotosintético en la hoja (clorofilas, carotenoides) extraídos en acetona acuosa, contenido de nitrógeno total (N) y valores de fluorescencia de clorofila Fv/Fm con las lecturas de SPAD-502 en hojas de sicomoro (*Acer pseudoplatanus*), haya (*Fagus sylvatica*) y encino inglés (*Quercus robur*) mostrando síntomas visuales de deficiencia de N. Independiente de las especies, se registraron altas correlaciones entre las lecturas de SPAD, clorofila total de la hoja y contenido de carotenoides, contenido de N foliar y valores Fv/Fm de fluorescencia de clorofila foliar; sin embargo, se obtuvo una pobre correlación entre los valores de SPAD y relaciones clorofila total:carotenoides. Las lecturas menores a 25 de SPAD en las tres especies indicaron afectación del proceso fotosintético que a su vez estuvo correlacionado con un contenido de N foliar <1.5%. Los resultados de este estudio indican que el medidor del contenido de clorofila SPAD-502 ofrece potencialmente un útil sistema no-destructivo para ayudar en la evaluación de la salud del árbol. Sin embargo, los usuarios deben estar alertas de las limitaciones de este sistema. Se puede necesitar consistencia en la colección de muestras y época estacional para especies y la calibración de ecuaciones de cultivares para correlacionar los valores de SPAD con las reducciones en la vitalidad del árbol.