PHOTOSYNTHETICA 40 (2): 289-292, 2002

The use of a portable non-destructive type nitrogen meter for leaves of woody plants in field studies

T. ICHIE*, Y. KITAHASHI**, S. MATSUKI**, Y. MARUYAMA***, and T. KOIKE*

*Hokkaido University Forests, FSC, Kita-9, Nishi-9, Kita-ku, Sapporo 060-0809, Japan**
*Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan***
*Forest and Forest Products Research Institute, Sapporo, 062-8516, Japan****

Abstract

The practicality of the portable, non-destructive type nitrogen meter (*Agriexpert PPW-3000*) was tested on ten forest species. Also investigated was the potential relationship between leaf nitrogen and chlorophyll (Chl) contents and the readings taken with the *PPW-3000* and a Chl meter (*SPAD-502*). There was a significantly positive correlation between the readings of *PPW-3000* and N content in the same leaves, whereas the correlation between leaf Chl content and the *PPW-3000* values was less positive. Similarly there was a significant positive correlation between actual Chl content and the *SPAD-502* readings and the less positive correlation between actual N content and the *SPAD-502* readings. Thus using both the *PPW-3000* and *SPAD-502* enables to determine leaf N and Chl contents simply and non-destructively in the field.

Additional key words: *Agriexpert PPW-3000*; chlorophyll; comparison of methods, *SPAD-502*.

Introduction

In natural ecosystems, nitrogen is a major nutrient that regulates plant growth (*e.g.* Field and Mooney 1986, Lambers *et al.* 1998). Its content is positively correlated with photosynthetic capacity, productivity, and consequently plant biomass (*e.g.* Field and Mooney 1986, Sage and Pearcy 1987, Evans 1989, Körner 1989, Reich *et al.* 1991, 1992, Gower *et al.* 1993, Pons *et al.* 1994, Hikosaka and Hirose 2000). Moreover, leaf N content changes with leaf expansion (Gastal *et al.* 1992), leaf ageing (Hodáňová 1985, Šesták 1985, Kutík *et al.* 2001), and leaf life span (Reich *et al.* 1992, 1995). Thus, if leaf N content can be measured easily and non-destructively for various plant species and individual trees in the field, its determination will undoubtedly be useful for better understanding the growth conditions and allocation strategies during plant life.

Although there have been many methods established for N analysis (*e.g.* Horwitz 2000), these methods suffer from two apparent limitations: they are destructive and particularly time-consuming, especially in ecophysiological studies in which a large number of samples is handled. In recent years, leaf chlorophyll (Chl) content is often correlated with leaf N content, using a portable, non-destructive Chl meter, the *SPAD-502* (*e.g.* Ishida *et al.* 1996, Manetas *et al.* 1998). However, few studies have measured leaf N directly in the field. Instead, methods involving calculation of leaf N content indirectly from substitute sources such as the readings from a Chl meter and a leaf colour chart have been used (*e.g.* Balasubramanian *et al.* 1999, Madakadze *et al.* 1999, Sandoval-Villa *et al.* 1999, Carreres *et al.* 2000, Rodriguez and Miller 2000). The recently introduced, portable nitrogen meter (*Agriexpert PPW-3000*), developed for rice plants, provides an excellent means of quickly and non-destructively assessing N content in leaves in the field. Because rice plants have a unique leaf blade structure (Esau 1965, Hara 1981, Chonan 1983), the question of how useful a N meter is when measuring content for several tree species, particularly in relation to optical structure of their leaves, has to be answered.

This study examined the usefulness of the *PPW-3000* for woody plants and also discussed the correlation between leaf N and Chl readings measured by the *PPW-3000* and the *SPAD-501*, respectively.

Received 28 February 2002, *accepted* 27 June 2002.
Fax: +81-11-706-3450; e-mail: ichie@fsc.hokudai.ac.jp
Acknowledgements: This research was partly supported by the Japan Science and Technology Agency, MEXT-JSPS (No. 14360078), and JSPS Research Fellowships for Young Scientists (T.I.).

Materials and methods

Ten forest species used for the investigation grow in Japanese temperate and Malaysian tropical rain forests with a great species diversity and, as a result, various types of leaves. Our study included: four deciduous broad-leaved trees from the arboretum of the Forestry and Forest Products Research Institute, Sapporo, Japan (42°58' N, 141°24' E) and Mount Chokai, Japan (39°05' N, 140°04' E), three temperate evergreen broad-leaved trees from the Mount Matsuyama Castle, Japan (33°50' N, 132°46' E), and three tropical evergreen broad-leaved trees from Lambir Hills National Park, Sarawak, Malaysia (4°20' N, 113°50' E) (Table 1). For each species, more than fifteen leaves of various green colour including leaves from trees of shaded and open sites were collected by three individuals and were wrapped in air tight plastic bags with moistened filter paper. Once collected, they were transferred immediately to the lab.

From all leaves two leaf discs (0.5 cm^2 per disc) were collected. One disc was used for N analysis, the other for Chl analysis. Then readings for all discs were taken with an *Agriexpert PPW-3000* (*Satake Corp.*) portable N meter and an *SPAD-502* (*Minolta*) portable Chl meter. *Agriexpert PPW-3000* measures leaf transmittance at four wavelengths by light-emitting diodes (LED), *i.e.* at 560 nm where is the peak of leaf reflection and permeation, and absorption of carotenoids and anthocyanin, at 660 nm where Chl absorbs (Šesták 1985, Lei *et al.* 1996), at 900 nm where some proteins with methyl group absorb (Osborne and Fearn 1986), and at 950 nm which is related with water absorption for base line, respectively. However, the measurement of *SPAD-502* was based on the comparison of leaf transmittance at two wavelengths, *i.e.* at 650 and 940 nm (Manetas *et al.* 1998).

Actual N content was analysed by the *N.C.* analyser (*SUMIGRAPH NC-900*). Chl was extracted with dimethylsulfoxide (Barnes *et al.* 1992, Shinano *et al.* 1996). For the Chl analysis, absorbances at 664.9 and 648.2 nm were determined by a *Shimazu UV-1400* spectrophotometer. The significance levels for correlation between N and Chl contents and the readings with *PPW-3000* and *SPAD-502* were calculated by multiple regression (independent variable readings of each meter at 560, 660, 880, 950 nm, respectively, 650 and 950 nm, subordination variable: N and Chl contents analysed by standard methods) using a *Statistica 5.1* statistical software package.

Table 1. Relationship between nitrogen contents measured by standard analysis and by *Agriexpert PPW-3000*. LED1: 950 nm, LED2: 660 nm, LED3: 900 nm, LED4: 560 nm. NS – not significant ($p>0.05$), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. D – deciduous, E – evergreen. n – number of leaves taken from three individuals. SLA – specific leaf area [$m^2\ kg^{-1}$], means ± S.D. The range of N contents [%] was calculated from values measured by the *N.C.* analyser.

Species	Family	Leaf type	n	SLA	N contents	r		Approximate expression
Fraxinus mandshurica	Oleaceae	D	19	15.2±4.6	1.83-2.77	0.796	***	y = 0.28 + 19.48 LED1 + 1.72 LED2 – 29.38 LED3 + 5.31 LED4
Acer mono	Aceraceae	D	17	24.2±9.2	1.67-3.00	0.762	**	y = 4.79 – 215.70 LED1 + 2.76 LED2 + 212.16 LED3 – 14.49 LED4
Quercus mongolica	Fagaceae	D	16	14.0±4.4	1.46-2.29	0.846	***	y = 1.36 + 98.08 LED1 – 3.97 LED2 – 151.88 LED3 + 55.80 LED4
Fagus crenata	Fagaceae	D	15	13.1±3.3	1.56-2.19	0.779	*	y = 1.65 – 3.44 LED1 + 0.27 LED2 – 3.51 LED3 + 4.43 LED4
Quercus glauca	Fagaceae	E	15	9.9±3.2	1.16-1.91	0.842	**	y =-0.01 – 95.12 LED1 – 2.48 LED2 + 64.93 LED3 + 33.65 LED4
Ilex latifolia	Aquifoliaceae	E	15	7.2±0.4	0.92-1.30	0.852	**	y = 1.63 – 25.38 LED1 + 2.39 LED2 + 45.73 LED3 – 25.15 LED4
Ilex integra	Aquifoliaceae	E	15	13.4±3.1	1.05-1.76	0.911	**	y = 5.95 – 91.51 LED1 + 2.05 LED2 + 105.49 LED3 – 27.32 LED4
Dryobalanops aromatica	Dipterocarpaceae	E	32	5.7±0.6	1.01-1.63	0.808	***	y = 2.42 – 31.80 LED1 + 1.02 LED2 + 24.62 LED3 – 1.04 LED4
Dipterocarpus tempehes	Dipterocarpaceae	E	15	7.4±1.5	1.67-2.19	0.994	***	y = 1.91 – 14.76 LED1 + 0.43 LED2 + 6.63 LED3 + 3.56 LED4
Shorea beccariana	Dipterocarpaceae	E	15	5.0±0.5	1.01-1.31	0.594	NS	y = 2.04 – 22.91 LED1 + 1.28 LED2 + 42.09 LED3 – 20.90 LED4

Results and discussion

For all tree species, the values of leaf N content estimated using the *PPW-3000* showed a positive and significant correlation with actual values determined by a standard method (Table 1). Thus *PPW-3000* is useful not only for rice plants, but also for woody species. However, some low correlations were found due to low transmittance in plant species such as *Shorea beccariana*. Therefore, we need a prior check whether *PPW-3000* can be used for a given species. N and Chl distribution in leaves is not uniform, but varies with leaf development and location in plant canopy (Šesták 1985). Therefore, one has to be careful when using the *PPW-3000* and consider the sampling location of the leaves, which live at different irradiances, and also select the types and periods of leaf emergence such as flush type, continuous type, or heterophyllous type. All the above considerations, as well as making sure to respect various N contents, improve the precision of determination.

Table 2. The correlation coefficients (R) of leaf nitrogen and total chlorophyll (Chl) between readings of *Agriexpert PPW-3000* and *SPAD-502*, and standard analyses. NS – not significant ($p>0.05$), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Species	Nitrogen				Chl			
	Agriexpert		*SPAD*		*Agriexpert*		*SPAD*	
Fr. mandshurica	0.796	***	0.779	***	0.745	***	0.809	***
A. mono	0.762	**	0.460	NS	0.960	***	0.884	***
Q. mongolica	0.846	***	0.494	NS	0.679	**	0.813	***
F. crenata	0.779	*	0.693	**	0.979	***	0.906	***
Q. glauca	0.842	**	0.727	**	0.746	*	0.850	***
I. latifolia	0.852	**	0.757	**	0.809	**	0.757	**
I. integra	0.911	**	0.505	NS	0.798	**	0.948	***
Dr. aromatica	0.808	***	0.568	***	0.926	***	0.940	***
D. tempehes	0.994	***	0.949	**	0.864	*	0.920	**
S. beccariana	0.594	NS	0.469	NS	0.847	***	0.899	***

The correlation of real leaf N content with the readings of *PPW-3000* was significantly more positive than with readings from the *SPAD-502* (Table 2). However, the correlation of leaf Chl content with the *SPAD-502* readings was significantly more positive than its correlation with the *PPW-3000* readings. Although *SPAD-502* measures at 650 nm where Chl absorbs radiation, *PPW-3000* measures at other two wavelengths related with leaf N. When calculating leaf N content from *SPAD-502*, one must consider that the relationship between the reading and the actual leaf N content varies with growth stage (Carreres *et al.* 2000). The species, for which the correlation was not significant, when recorded with the *SPAD-502*, were *Acer mono, Qercus mongolica,* and *Ilex integra*, all of which showed a significant correlation when using *PPW-3000*. The readings at each wavelengths for the *PPW-3000* were not in significant correlation with leaf N content, which generally showed a significant correlation with the *PPW-3000* readings (Table 3). Thus the *PPW-3000* is useful especially for the species lacking correlation between Chl absorptance and leaf N. Our results indicate that using together the *PPW-3000* and the *SPAD-502* enables to measure both leaf N and Chl contents simply, rapidly, and non-destructively in the field. Non-destructive methods are optimal for field studies such as N distribution in the crown, acclimation to shade (*i.e.* Chl/N), and seasonal changes of leaf N content at species, population, or community level.

Table 3. Significant differences of correlation between readings of four wavelengths (LED) of *Agriexpert PPW-3000* and *SPAD-502*, and nitrogen contents measured by the *N.C.* analyser. NS – not significant ($p>0.05$), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

	Fr. mandshurica	*A. mono*	*Q. mongolica*	*F. crenata*	*Q. glauca*	*I. latifolia*	*I. integra*	*Dr. aromatica*	*D. tempehes*
Agrixepert	***	**	***	*	**	**	**	***	***
LED1	NS	**	NS	NS	NS	NS	*	NS	*
LED2	**	NS	NS	*	**	**	NS	**	***
LED3	NS	**	NS	NS	NS	NS	NS	NS	*
LED4	NS	NS	NS	NS	*	NS	NS	NS	***
SPAD	***	NS	NS	**	**	*	NS	***	**

References

Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S., Davison, A.W.: A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. – Environ. exp. Bot. **32**: 85-100, 1992.

Balasubramanian, V., Morales, A.C., Cruz, R.T., Abdulrachman, S.: On-farm adaptation of knowledge-intensive nitrogen management technologies for rice systems. – Nutr. Cycl. Agroecosyst. **53**: 59-69, 1999.

Carreres, R., Sendra, J., Ballesteros, R., García De La Caudra, J.: Effects of preflood nitrogen rate and midseason nitrogen timing on flooded rice. – J. agr. Sci. **134**: 379-390, 2000.

Chonan, N.: [Structure and function of leaves in relation to photosynthate translocation in Graminoides.] – Jap. J. Crop Sci. **52**: 379-385, 1983. [In Japan.]

Esau, K.: Plant Anatomy. 2nd Ed. – John Wiley & Sons, New York 1965.

Evans, J.R.: Photosynthesis and nitrogen relationship in leaves of C_3 plants. – Oecologia **78**: 9-19, 1989.

Field, C.B., Mooney, H.A.: The photosynthesis-nitrogen relationship in wild plants. – In: Givnish, T.J. (ed.): On the Economy of Plant Form and Function. Pp. 25-55. Cambridge University Press, Cambridge – London – New York – New Rochelle – Melbourne – Sydney 1986.

Gastal, F., Belanger, G., Lemaire, G.: A model of the leaf extension rate of tall fescue in response to nitrogen and temperature. – Ann. Bot. **70**: 437-442, 1992.

Gower, S.T., Reich, P.B., Son, Y.: Canopy dynamics and aboveground production of five tree species with different leaf longevities. – Tree Physiol. **12**: 327-345, 1993.

Hara, N.: [Plant Form: Stem · Leaf · Root · Flower.] – Baufukan, Tokyo 1981. [In Japan.]

Hikosaka, K., Hirose, T.: Photosynthetic nitrogen-use efficiency in evergreen broad-leaved woody species coexisting in a warm-temperate forest. – Tree Physiol. **20**: 1249-1254, 2000.

Hodáňová, D.: Leaf optical properties. – In: Šesták, Z. (ed.): Photosynthesis During Leaf Development. Pp. 107-127. Academia, Praha; Dr W. Junk Publ., Dordrecht – Boston – Lancaster 1985.

Horwitz, W.: Official Methods of Analysis of AOAC International. 17th Ed. – AOAC International, Gaithersburg 2000.

Ishida, A., Toma, T., Matsumoto, Y., Yap, S.K., Maruyama, Y.: Diurnal changes in leaf gas exchange characteristics in the uppermost canopy of a rain forest tree, *Dryobalanops aromatica* Gaertn. f. – Tree Physiol. **16**: 779-785, 1996.

Körner, C.: The nutritional status of plants from high altitudes. A worldwide comparison. – Oecologia **81**: 379-391, 1989.

Kutík, J., Holá, D., Vičánková, A., Šmídová, M., Kočová, M., Körnerová, M., Kubínová, L.: The heterogeneity of structural and functional photosynthetic characteristics of mesophyll chloroplasts in various parts of mature or senescing leaf blade of two maize (*Zea mays* L.) genotypes. – Photosynthetica **39**: 497-506, 2001.

Lambers, H., Chapin, F.S., III, Pons, T.L.: Plant Physiological Ecology. – Springer-Verlag, New York – Berlin – Heidelberg 1998.

Lei, T.T., Tabuchi, R., Kitao, M., Koike, T.: Functional relationship between chlorophyll content and leaf reflectance, and light-capturing efficiency of Japanese forest species. – Physiol. Plant. **96**: 411-418, 1996.

Madakadze, I.C., Stewart, K.A., Madakadze, R.M., Peterson, P.R., Coulman, B.E., Smith, D.L.: Field evaluation of the chlorophyll meter to predict yield and nitrogen concentration of switchgrass. – J. Plant Nutr. **22**: 1001-1010, 1999.

Manetas, Y., Grammatikopoulos, G., Kyparissis, A.: The use of the portable, non-destructive, SPAD-502 (Minolta) chlorophyll meter with leaves of varying trichome density and anthocyanin content. – J. Plant Physiol. **153**: 513-516, 1998.

Osborne, B.G., Fearn, T.: Near Infrared Spectroscopy in Food Analysis. – Pp. 184-193. Longman Scientific & Technical, New York 1986.

Pons, T.L., Van der Werf, A., Lambers, H.: Photosynthetic nitrogen use efficiency of inherently slow- and fast-growing species: possible explanations for observed differences. – In: Roy, J., Garnier, E. (ed.): A Whole-Plant Perspective of Carbon-Nitrogen Interactions. Pp. 61-77. SPB Academic Publishing, The Hague 1994.

Reich, P.B., Koike, T., Gower, S.T., Schoettle, A.W.: Causes and consequences of variation in conifer leaf life-span, – In: Smith, W.K., Hinckley, T.M. (ed.): Ecophysiology of Coniferous Forests. Pp. 225-254. Academic Press, San Diego 1995.

Reich, P.B., Uhl, C., Walters, M.B., Ellsworth, D.S.: Leaf lifespan as a determinant of leaf structure and function among 23 Amazonian tree species. – Oecologia **86**: 16-24, 1991.

Reich, P.B., Walters, M.B., Ellsworth, D.S.: Leaf lifespan in relation to leaf, plant, and stand characteristics among diverse ecosystems. – Ecol. Monogr. **62**: 365-392, 1992.

Rodriguez, I.R., Miller, G.L.: Using a chlorophyll meter to determine the chlorophyll concentration, nitrogen concentration, and visual quality of St. Augustinegrass. – HortScience **35**: 751-754, 2000.

Sage, R.F., Pearcy, R.W.: The nitrogen use efficiency of C_3 and C_4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). – Plant Physiol. **84**: 959-963, 1987.

Sandoval-Villa, M., Wood, C.W., Guertal, E.A.: Ammonium concentration in solution affects chlorophyll meter readings in tomato leaves. – J. Plant Nutr. **22**: 1717-1729, 1999.

Šesták, Z.: Chlorophylls and carotenoids during leaf ontogeny. – In: Šesták, Z. (ed.): Photosynthesis During Leaf Development. Pp. 76-106. Academia, Praha; Dr W. Junk Publ., Dordrecht – Boston – Lancaster 1985.

Shinano, T., Lei, T.T., Kawamukai, T., Inoue, M.T., Koike, T., Tadano, T.: Dimethylsulfoxide method for the extraction of chlorophylls *a* and *b* from the leaves of wheat, field bean, dwarf bamboo, and oak. – Photosynthetica **32**: 409-415, 1996.