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Inherent Limitations of Nondestructive Chlorophyll Meters: A Comparison of Two Types of Meters

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Abstract. **Two types of nondestructive chlorophyll meters were compared with a standard, destructive chlorophyll measurement technique. The nondestructive chlorophyll meters were 1) a custom built, single-wavelength meter, and 2) the recently introduced, dual-wavelength, chlorophyll meter from Minolta (model SPAD-502). Data from both meters were closely correlated with destructive measurements of chlorophyll (r^2 = 0.90 and 0.93; respectively) for leaves with chlorophyll concentrations ranging from 100 to 600 mg·m^{-2}, but both meters consistently overestimated chlorophyll outside this range. Although the dual-wavelength meter was slightly more accurate than the single-wavelength meter (higher r^2), the light-scattering properties of leaf cells and the nonhomogeneous distribution of chlorophyll in leaves appear to limit the ability of all meters to estimate in vivo chlorophyll concentration.**

Leaf chlorophyll content is often well correlated with leaf N status, RuBP carboxylase activity, and photosynthetic capacity (Evans, 1983; Seemann et al., 1987). Chlorophyll content is also a sensitive indicator of many types of plant stress (Palta, 1990) and can be used to select for acclimation and tolerance to low temperature (Eagles et al., 1983).

Standard in vitro colorimetric methods for chlorophyll determination are reasonably accurate (± 5%) but are time consuming and destructive. Chlorophyll concentration can also be measured in vivo by nondestructive spectroscopic techniques that do not require sample preparation. Two approaches have been used to estimate chlorophyll content in vivo: reflectance measurements (Gausman, 1985; Singha and Townsend, 1989) and absorbance measurements at 676 nm. Reflectance measurements, which have been used primarily in remote sensing, are complicated by leaf thickness and leaf water content and require dust-free leaf surfaces (Maas and Dunlap, 1989; Macnicol et al., 1976). Several designs for building in vivo chlorophyll meters based on absorbance have been published (Eagles et al., 1983; Hardacre and Nicholson, 1984; Macnicol et al., 1976), and a new commercial meter is now available (mode1 SPAD-502, Minolta Corp., Ramsey, N.J.; Minolta, 1989).

The objective of this study was to evaluate a custom-built, single-wavelength meter and the Minolta dual-wavelength chlorophyll meter (model SPAD-502). We wanted to determine if a less expensive, single-wavelength meter would adequately measure chlorophyll concentration. Chlorophyll was measured in three ways: 1) nondestructive-single-wavelength meter (in vivo); 2) nondestructive-dual-wavelength meter (in vivo); and 3) destructive-calorimetric measurement (in vitro).

The single-wavelength sensor and associated electronics were developed in our laboratory following the principles described in Hardacre and Nicholson (1984), who reviewed several designs and recommend the use of solid-state components (because of their thermal stability) and light-emitting diodes (LEDs) because they are cold sources of light. LEDs allow the light source to be placed closer to the leaf, which makes the instrument more portable and reduces power consumption. The detector should also be placed as close as possible to the leaf so that it can view a large angle, thus reducing the effects of light scattering (see discussion).

The single-wavelength meter measures the intensity of the light transmitted through the leaf between 610 and 680 nm using a red LED (AND Co., Burlingame, Calif.; model AND154UR), a Schott RG610 longpass filter (Oriel Corp., Stratford, Conn.), and a gallium arsenide photodiode sensor (Hamamatsu, San Jose, Calif.; model G1736), which is not sensitive beyond 680 nm. The sensor has a photosensitive area of 7.6 mm^2 and is placed as close as possible to the leaf. A current-to-voltage converter circuit amplifies the signal, which is then displayed on a digital panel meter (Texmate, Solana Beach, Calif.; mode1 RP-35A).

The nondestructive, dual-wavelength meter was a Minolta SPAD-502. The measuring head of the SPAD-502 includes a red LED and an infrared LED, which emit light in sequence through the leaf. The SPAD-502 is self-calibrating for variability in the output of the LEDs and has built-in error codes that help to prevent irregular measurements. Peak chlorophyll absorbance is measured at 650 nm, and nonchlorophyll absorbance (cell walls, etc.) is measured at 940 nm. A microprocessor calculates a SPAD value, which is proportional to the relative optical density, based on the ratio between the two wavelengths (Minolta, 1989). This meter was calibrated using the reading checker supplied by the manufacturer, which standardizes the SPAD readings among different SPAD-502 meters, as well as making its readings comparable to SPAD-501 values. This calibration procedure was first used for the SPAD-501, a predecessor of the SPAD-502, by Marquard and Tipton (1987).

Destructive colorimetric measurement was made using the dimethyl sulfoxide (DMSO) extraction procedure (Hiscox and Israelstam, 1979), results for which were virtually identical to measurements with the standard 80% aqueous acetone extraction, confirming previous studies (Hains, 1985; Hiscox and Israelstam, 1979). Leaf disks (1 to 3 cm^2) were taken from three species {rice *(Oryza sativa* L.), soybean *[Glycine max* (L.) Merr.], and

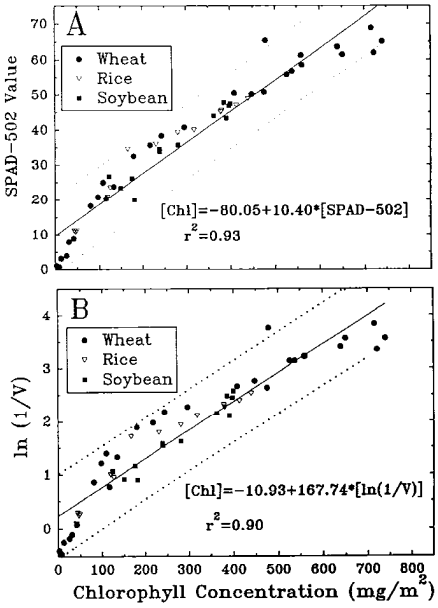

Fig. 1. (**A**) Relationship between the dual-wavelength chlorophyll value and leaf chlorophyll concentration in three species. Measurements were made with a Minolta portable chlorophyll meter (model SPAD-502). This meter electronically corrects absorption at a reference wavelength (940 nm) and linearizes the signal. (**B**) The relationship between negative natural logarithm of output voltage from the single-wavelength sensor and chlorophyll concentration. The sensor and associated electronics were developed in our laboratory. It is necessary to determine the negative natural logarithm of the sensor output voltage because light is exponentially attenuated as it passes through a medium (Beer's Law).

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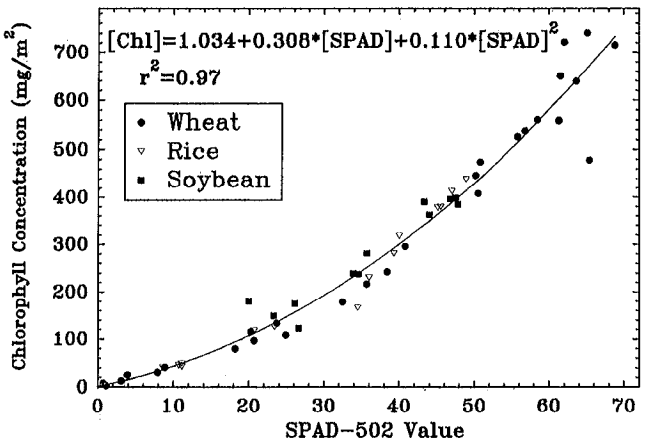

Fig. 2. A nonlinear fit provided a better correlation between chlorophyll concentration and SPAD-502 values (r^2 = 0.97) than a linear one.

wheat *(Tritium aestivum* L.)} with a #3 cork borer and weighed. Leaf disk area was measured with a leaf area meter (LI-COR, Lincoln, Neb.; model LI-3000), and replicate readings of area were within ± 4%. About 20 to 40 mg of leaf material was placed in opaque screw-cap vials containing 10 ml of DMSO and incubated in an oven for 3 h at 65C. Three milliliters of the resulting extract were placed in a quartz cuvette, and absorbance at 710, 663, and 645 nm was measured in a Beckman (Beckman, Fullerton, Calif.) spectrophotometer. Chlorophyll concentration was calculated using the equations from Porra et al. (1989). These equations are an improvement over the widely used equations of Arnon (1949). A wide range of leaf chlorophyll concentrations was selected by using senescing, typical, and well-fertilized dark-green leaves from greenhouse-grown plants.

The area of the leaf sampled was an important factor in the accuracy and reproducibility of both meters. Veins are relatively transparent and chlorophyll concentration is not homogeneous, so measurements in adjacent leaf sections (a few millimeters apart) often varied up to 50%. The leaf area measured by the dual-wavelength meter was 6 m m^2; that measured by the single-wavelength meter was 7.6 mm^2. Because of this difference, six separate measurements on each leaf disk had to be taken to obtain an average value for comparison with the destructive chlorophyll measurement. The minimum leaf area used for the destructive measurement was 1 to 3 cm^2 because smaller areas were difficult to measure and the amount of chlorophyll was too small for accurate measurement, especially in chlorotic leaves. Although larger leaf samples would have better represented the average chlorophyll content of the leaf, we used the smallest sample size possible to more closely approximate the area measured by the nondestructive meters.

The dual-wavelength meter produced only a slightly better correlation with the destructive measurement than the single-wavelength meter (r^2 = 0.93 vs. r^2 = 0.90; Fig. l), and the slopes of the regression lines were not significantly different ($P \leq 0.05$; Snedecor and Cochran, 1980). Individual measurements made with the dual-wavelength meter, however, were more repeatable and had a smaller coefficient of variation than those with the single-wavelength meter (54% vs. 67%, respectively). For this reason, it was more important to average multiple readings from the single-wavelength meter. In spite of the favorable r^2 relationships, nondestructive meters should be used with caution because some measurements varied up to 50% from the destructively measured chlorophyll concentration (note the 95% prediction intervals in Fig. 1).

Both meters consistently overestimated chlorophyll concentration in leaves with chlorophyll concentrations > 600 mg·m^{-2} or <100 mg·m^{-2} (Fig. 1). One of our most interesting findings was that both meters gave inaccurate readings in the same leaf samples (the coefficient of determination for the relationship of the two meters was r^2 = 0.98).

Beer's Law predicts that the relationship between in vivo and in vitro measurements would be linear if absorbance were solely dependent on pigment concentration. However, the transmission of light through a leaf is determined not only by pigment concentration, but also by light scattering, leaf surface reflectivity, and pigment distribution in the leaf (Vogelmann, 1989). A combination of these morphological factors probably explains why the relationships between in vivo and in vitro measurements illustrated in Fig. 1 are curvilinear (Macnicol et al., 1976).

Light scattering is a function of the arrangement of cells within a leaf. Its effect is to reduce the amount of light transmitted by increasing the optical pathlength through the leaf (Vogelmann, 1986). The major light-scattering component in plants appears to be refraction at the interfaces between cells and air spaces (Terashima and Saeki, 1983; Vogelmann, 1989).

Single-wavelength meters can minimize the effects of light scattering by using LEDs with collimating lenses, by having a large detector area compared to the area of the light source, and by placing the detector as close as possible to the leaf (Hardacre and Nicholson, 1984). Dual-wavelength meters can further reduce these effects because scattering at a reference wavelength can be subtracted from the chlorophyll-absorbing wavelengths (Butler, 1964). However, reflectivity at the reference wavelength must be similar to the absorption wavelength because differences in reflectivity contribute to measurement error (Butler, 1964). Surface reflectivity effects are particularly significant in leaves with a low chlorophyll concentration because they typically have a higher reflectance (Gausman, 1985).

The dual-wavelength meter may not completely correct for scattering because leaf reflectance at 940 nm (the reference wavelength of the SPAD-502) is much higher than at 660 nm (the chlorophyll absorption wavelength; Maas and Dunlap, 1989). A reference wavelength in the visible range likely would more effectively correct for scattering. This scattering may explain why both meters have the same nonlinear response at lower chlorophyll concentrations.

Spatial pigment distribution also affects transmission because of the sieve effect, Absorption efficiency is reduced as pigment distribution becomes less uniform (Vogelmann, 1986). Terashima and Saeki (1983) suggest that chlorophyll is less uniformly distributed in high-chlorophyll leaves because there is an increase in chlorophyll density in chloroplasts rather than an increase in the number of chloroplasts. The sieve effect may explain why both nondestructive meters overestimated chlorophyll at concentrations > 650 mg·m^{-2}.

Environmental conditions affect leaf morphology, which in turn affects in viva/in vitro chlorophyll relationships. The equation in Fig. 2 quantifies the relationship between SPAD-502 values and chlorophyll for the species we used in our greenhouse environment; however, the equation may not be valid for other environments. Yadava (1986) obtained a poor correlation (r^2 of only 0.48) between nondestructive measurements and extracted chlorophyll for 22 species collected in diverse growth environments. Campbell et al. (1990) recently demonstrated that environment has a large effect on the in vivo/in vitro chlorophyll relationship.

In spite of these limitations, chlorophyll meters are valuable instruments if they are properly calibrated. Nondestructive meters must be calibrated with extractable chlorophyll measurements for a given set of growing conditions. Multiple readings should be averaged to obtain a representative value for a leaf section. Although single measurements can be inaccurate, multiple readings and average measurements can be made in far less time than with destructive measurements. The dual-wavelength meter was not significantly better than the single-wavelength meter, but the commercial meter facilitated averaging and editing stored values.

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A Pulsed Subirrigation System for Small Plots

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Abstract. **A schematic diagram and parts list is presented for a simple and inexpensive system for pulsed subirrigation (commerically referred to as ebb and flow or flood and drain). The system can be readily modified for flowing solution culture. It has proven useful in teaching and research applications. It can be assembled from readily available parts using hand tools.**

Intermittent or pulsed subirrigation systems (commercially referred to as ebb and flow, or flood and drain) have substantial advantages in greenhouse crop production, including the potential to significantly reduce runoff of water and fertilizers (Elliott, 1990). Research is needed to provide fertilizer recommendations and to investigate media-fertilizer interactions relevant to crop production using this method. As these systems are being widely adopted in the industry, students of greenhouse management and crop production need to learn the principles of pulsed subirrigation in greenhouse operations.

Commercially pulsed irrigation systems are available, but these systems may be difficult to use in research and teaching applications. Because of the size and expense of these systems, it is difficult to provide for true replication of treatments in which the basic unit of observation (research plot) involves factors that require separate irrigation capabilities, such as fertilizer composition, growing medium, or crop. For these reasons, I have developed a simple, modular system for small-plot pulsed subirrigation experimental units.

In this system (Fig. 1), the basic unit comprises a tray to which a single treatment is assigned. Irrigation solution is pumped into the tray by a centrifugal pump from a reservoir. The level of solution in the tray is regulated by an overflow outlet. When the pump shuts off, the solution returns to the reservoir via the inlet line. The tray can be supported by a frame that allows the inlet and return lines to be fitted, or can rest directly on the reservoir.

The tray (parts list item 1) is a commercial nursery flat, without drainage holes, made of structural foam plastic (Kadon Corp., Dayton, Ohio, mode1 2014-4-P). The structural foam is thick enough (6.5 mm) to drill and tap for a threaded fitting (parts list 14). The inlet fitting must be flush with the inner surface of the tray, so that the tray drains completely at the end of the irrigation cycle. An earlier model, similar to the microcosm proposed for toxicity testing by the Environmental Protection Agency (Federal Register, 1987), used a thinner tray with the inlet fitting welded or glued to the underside of the tray, but these tended to leak or break off. A hole saw is used to cut an opening for a bulkhead fitting (parts list 2) for the overflow return line. Either a single or dual thread bulkhead fitting can be used. The dual thread has the advantage of allowing the use of a threaded fitting (parts list 3) as a standpipe to adjust the solution height in the tray. Without a standpipe, the solution will rise to -1 cm. If this flexibility is not required, a single thread fitting is less cumbersome and less expensive.

The irrigation solution (water or nutrient solution) is contained in a reservoir (parts list 8). I have found that » 8 to 10 liters/tray is required to fill the tray to a depth of 1 cm while maintaining enough solution in the reservoir to keep the pump submerged. A cover to reduce evaporation and contamination is useful but not essential. Solution can be added to the reservoir manually or with an automatic fertilizer proportioner. The solution is pumped into the tray by a small submersible centrifugal pump (parts list 9; Little Giant Pump Co., Oklahoma City, Okla., model NK-1). The pump can be controlled by a spring-wound appliance timer (M.H. Rhodes, Inc., Avon, Conn., model 78305) or a conventional repeat cycle time clock. Solution inlet flow rate can be controlled with a quarter-turn valve (parts list 15; Gilmour Mfg., Somerset, Pa., model 07V). Depending on the physical relation of the tray and reservoir, it may be more convenient to locate the valve near the point at which the inlet supply line leaves the reservoir.

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