Summary
We conducted two experiments to determine the usefulness of a chlorophyll content meter (CCM) for the measurement of foliar chlorophyll concentration in sugar maple (Acer saccharum Marsh.) in the fall color period. In Experiment 1, four sugar maple trees were visually assigned to each of four fall foliage color categories in October 1998. On four dates in the fall of 1999, leaves were taken from the trees and analyzed for chlorophyll concentration by absorbance of pigment extracts and by determination of the chlorophyll content index (CCI) with a CCM. The two measures of chlorophyll concentration were strongly correlated ($P < 0.001$, $r^2 = 0.72$).

In Experiment 2, the CCI of leaves from sugar maple trees subjected to one of four fertilization treatments (lime, lime + manure, lime + 10:10:10 N,P,K fertilizer and an untreated control) were determined with a CCM. Treatment effects were distinguishable between all pairwise comparisons ($P < 0.001$), except for the lime versus lime + NPK fertilizer treatments.

Keywords: CCI, CCM, nondestructive testing, spectrophotometry.

Introduction
Chlorophyll content meters (CCMs) have been used for several years by agronomists studying maize (Tenga et al. 1989, Dwyer et al. 1994, Chapman and Barretto 1997). They have the advantages of being pocket-portable, inexpensive, nondestructive to leaf tissue, rapid and easy to use, and do not require hazardous compounds or specially trained personnel. Data acquired can be rapidly downloaded for computer-based analysis.

Typical CCMs operate by differential absorption of light at two wavelengths, one in the near infrared that passes through leaf pigments relatively unimpeded and serves as a reference beam, and one tuned to the peak absorbance of chlorophyll. The transmission of beam energy, expressed as the ratio of absorbance beam to the standardized infrared reference beam, gives the chlorophyll content index (CCI), usually in the range of 1.0 (no pigment) to about 70.0 (very high pigment content), depending on instrument make and model.

Values of CCI are strongly correlated to extractable chlorophyll in annual crops like cotton, wheat fescue, rice, soy and maize (Marquand and Tipton 1987, Dwyer et al. 1994, Chapman and Barretto 1997). But there are few reports on the use of CCMs with broad-leaved trees (Campbell et al. 1990, Sibley et al. 1996). Our objective was to determine the utility of a CCM for the nondestructive determination of chlorophyll concentration in sugar maple (Acer saccharum Marsh.) leaves expressing a range of colors, and for the detection of changes in chlorophyll concentration in trees subjected to different soil amendments.

Materials and methods
Chlorophyll content meter
Leaf greenness was determined with a prototype of the Opti-Sciences CCM-200 chlorophyll content meter (Opti-Sciences, Tyngsboro, MA). This instrument uses differential transmission at two wavelengths, 940 and 665 nm, to determine the absorbance of chlorophyll pigments (whereas the current production model uses a 655 nm absorbance beam). Beam energy is sampled pre- and post-transmission, and the 940 nm reference beam is standardized according to the manufacturer’s instructions. The ratio of the red absorbance beam to the standardized infrared reference beam gives the chlorophyll content index (CCI).

Beams are generated by LEDs, and the leaf tissues and pigments are unharmed by sampling; the region measured by the sensing head is a circle 0.71 cm$^2$ in area (9.5 mm diameter).

Experiment 1
In Experiment 1, sugar maple trees growing at the USDA Forest Service Northeastern Research Station in South Burlington, VT, USA, were assigned to one of four fall foliage color categories based on their color in October 1998 (Schafer et al. 2003). The categories were established on the basis of a two-person visual assessment of whole-tree color character and number of leaves remaining.

Whole, lower-canopy branch segments were taken from four trees in each category weekly from September 5 to October 13, 1999. Branch segments were transported to the labora-
tory in a cooler in sealed plastic bags.

The CCI was sampled on five leaves from each branch segment with the CCM sensing head held as close as possible to the junction of the central vein and the next adjacent major vein without including major vein tissue under the device. Five non-overlapping measurements were taken on each leaf over homogeneous, healthy leaf tissue, and a mean value calculated from the measurements for each tree. In the course of the experiment, 16 trees were tested on four dates.

After the CCI had been sampled, five 6.4-mm diameter disks were punched from each leaf in the approximate locations of the CCI measurements. The disks were extracted in 80% (v/v) acetone at 4 °C in the dark. Transmittance of the extract was measured with a Spectronic-Unicam Genesis/8 spectrophotometer. Total chlorophyll was calculated according to Lichtenthaler and Wellburn (1983).

**Experiment 2**

In Experiment 2, we tested the ability of the CCM-200 to detect the effects of four fertilizer treatments applied to sugar maple trees the previous summer. The trees were located in a progeny plantation at the Proctor Maple Research Center (PMRC) in Underhill Center, VT, USA. The plantation was established in 1960 from seedlings of several known mother trees chosen for their high sugar content. The fertilizer treatments were dolomitic lime (3360 kg ha$^{-1}$), dolomitic lime and 10:10:10 N,P,K fertilizer (3360 kg ha$^{-1}$ and 280 kg ha$^{-1}$, respectively), and lime and manure (3360 kg ha$^{-1}$ and 51 m$^3$ ha$^{-1}$, respectively). A fourth group of trees that received no amendment served as a control.

In September 1999, 10 dominant trees were randomly selected from each of the treatment and control plots. Branches less than 2 cm in diameter were taken from the top third of the south-facing crown with a pole pruner. Five visually healthy, green leaves were randomly chosen from the outermost leaves on each branch. Leaves from trees in different treatments were visually indistinguishable. The CCI was measured as described for Experiment 1.

**Statistical analysis**

Data were subjected to regression analysis and ANOVA using the JMP-IN and StatView software applications (SAS Institute, Cary, NC). For Experiment 2, pairwise comparisons were made by Bonferroni compensated tests.

**Results and discussion**

In Experiment 1, extractable chlorophyll was strongly correlated with CCI (Figure 1, $P < 0.001$, $r^2 = 0.72$, $n = 64$) across all tree categories and sampling dates. The same level of significance and correlation was found in previous studies using CCMs with annual crops (Campbell et al. 1990, Reeves et al. 1993, Dwyer et al. 1994, Ma and Dwyer 1997). Mean leaf chlorophyll concentration was 0.258 µg mm$^{-2}$ for all fall color categories, consistent with the value found by Liu et al. (1997).

In Experiment 2, all treatments yielded higher CCI values than the control. Significant differences in CCI were found for all pairwise comparisons (Figure 2, $P < 0.0001$, $n = 40$), except for the lime versus lime + NPK fertilizer treatment ($P < 0.48$).

Several studies have shown that the CCI exhibits a nonlinear plateau response at the upper extreme of the measurement range (Marquand and Tipton 1987, Dwyer et al. 1994, Chapman and Barretto 1997). However the CCI values measured in the present study did not reach this plateau level.

Angle of incidence and PAR irradiance have been shown to affect chloroplast distribution and angle (Haupt 1982), and CCI values are significantly affected by the incident irradiance, typically giving lower values at higher irradiances (Hoel and Solhaug 1998). For these reasons, CCM devices provide the most reliable and reproducible measurements when actinic PAR is low. Differences in leaf optical density, leaf anatomy and other factors necessitate species-specific regression models for the CCI–chlorophyll content relationship (Schaper and Chacko 1991).
In conclusion, CCI values were strongly correlated with chlorophyll concentration as determined by absorbance of extracted pigments, indicating that the instrument can be used in the field to monitor leaf chlorophyll concentration in sugar maple.

Acknowledgments

This work was partly supported by a grant from the U.S. Environmental Protection Agency. We thank the North American Maple Syrup Council for assistance in purchasing the spectrophotometer used in this study. Additional thanks to Dan Harkins of Opti-Sciences for the loan of the CCM-200 prototype and to Dr. William Currier and Tim Wilmot of the University of Vermont for their helpful comments and access to unpublished work. Special thanks to Dr. Paul Schaberg and Paula Murakami of the USDA Forest Service Northeastern Research Station and to Abby van den Berg for allowing access to the experimental trees and samples that part of this study is based on.

References


