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Note

Use of an inexpensive chlorophyll meter to predict nitrogen levels in leaf tissues of water hyacinth (*Eichhornia crassipes*)

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INTRODUCTION

Plant nitrogen content can strongly influence invertebrate herbivore performance (Mattson 1980), and its impact on insect biological control agents of aquatic and wetland plants is documented (Forno and Bourne 1985, Room and Thomas 1986, Heard and Winterton 2000, Wheeler 2001, Center and Dray 2010, Center et al. 2014, Coetzee et al. 2007). Tissue nitrogen is also an important indicator of plant health and can be a useful predictor of plant vigor and susceptibility to disease and pests (Loh et al. 2002). Hence, knowing nitrogen content may aid in determining establishment success of plants used in restoration programs, including those destined for aquatic and wetland sites. However, most techniques used to determine plant nitrogen content require destructive, lengthy, and expensive chemical analyses, which often preclude their use on a routine basis. A nondestructive procedure is needed that provides actual readings of tissue nitrogen on a near–real-time basis.

Techniques have been developed that allow real-time analysis of plant tissue chlorophyll levels. These include the use of hand-held meters that measure light transmittance of leaves in the 600 to 700–nm range. This allows a determination of the "greenness" of the plant, which has been shown to be proportional to chlorophyll content (Loh et al. 2002, Coste et al. 2010, Zhu et al. 2012, Shapiro et al. 2013). Because chlorophyll and nitrogen content are closely related, a rapid method of estimating nitrogen in plants is readily available (Schepers et al. 1992). In fact, Dray et al. (2012) used the SPAD-520 chlorophyll meter (Spectrum Technologies, Inc., Plainfield, IL) to demonstrate its utility in estimating nitrogen content in water hyacinth leaves in near real time with good results. However, the SPAD-520 meter is expensive, which may preclude its use on a routine basis. More recently, a chlorophyll meter has become available that has been shown to be highly accurate in predicting plant nitrogen content but considerably lower in cost relative to the SPAD-520 (atLeaf+ meter, FT Green LLC, Wilmington, DE; Zhu et al. 2012). The utility of using the atLeaf+ chlorophyll meter to predict leaf nitrogen content in aquatic plants has not been evaluated; hence, we tested its use to determine chlorophyll levels in *Eichhornia crassipes* (Mart.) Solms (water hyacinth) leaves, and assessed its predictability in estimating nitrogen content with the use of data collected during another experiment designed to examine temperature impact on water hyacinth planthopper (*Megamelus scutellaris* Berg; Hemiptera: Delphacidae).

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MATERIALS AND METHODS

The atLeaf+ meter was used to determine greenness (proportional to chlorophyll) of water hyacinth leaf tissues during an experiment designed to examine the influence of temperature on the survival of the water hyacinth planthopper (*M. scutellaris* Berg; unpub. data). Water hyacinth was grown in 36 19-L containers over an 8-wk period beginning June 18, 2014 with the use of a standard high nitrogen-modified Hoagland's nutrient solution with added chelated iron (20 mg L^{-1} N and 4 mg L^{-1} Fe) in water baths of 18, 25, and 30 C. The nutrient solution in each container was exchanged weekly beginning on July 16, 2014 to maintain available nitrogen for maximum plant growth during the study. Two replications were eliminated from the analyses because of zero to minimal plant growth at 18 C. Planthoppers were added after 2 wk of initial plant growth and the experiment was harvested after 8 wk. Variations in temperature regimes and numbers of surviving planthoppers resulted in plants with a relatively wide range of N content. Chlorophyll measurements were recorded from three randomly selected, completely unfurled leaves in the middle position of the whorl to reduce potential variation produced by leaf age. This was repeated on each experimental container. Chlorophyll readings were obtained from the lower to mid portion of the leaf away from the medial line with the use of the atLeaf+ meter. Readings were

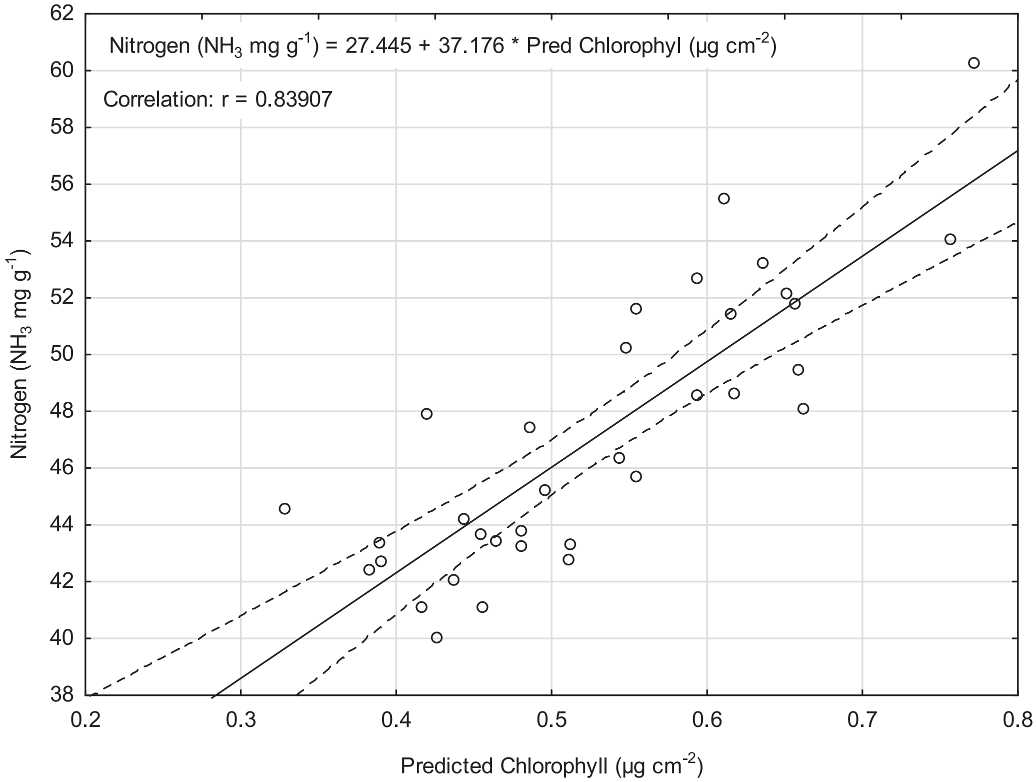

Figure 1. Linear relationship between predicted water hyacinth leaf chlorophyll levels based on atLeaf+ meter readings and actual water hyacinth above-water biomass nitrogen content. Relationships are significant at the $P < 0.05$ level.

obtained under relatively consistent ambient light levels. The three atLeaf+ readings from different leaves for each experimental container were averaged for each replication and the resulting mean used for the analyses. Unitless atLeaf+ values were converted to chlorophyll values with the use of regression equations averaged over several different crop species as presented by Zhu et al. (2012) as follows:

$$\text{predicted chlorophyll}\,(\mu\text{g cm}^{-2}) = (\text{atLeafValue} - 15.1)/52.4.$$

We could have compared atLeaf+ values directly to measured nitrogen levels and obtained similar results. However, because most researchers use chlorophyll as the basis for comparisons, we decided to follow the same protocol (Coste et al. 2010, Shapiro et al. 2013).

Nitrogen content in above-water biomass was determined for water hyacinth (leaves and petioles). During experimental harvest, tissues were weighed and dried at 60 C to a consistent weight in order to eliminate differences due to moisture content. A 250-mg subsample was then ground to a fine powder and subjected to a sulfuric acid/hydrogen peroxide block digestion (Allen et al. 1974) and held at 4 C until being analyzed for nitrogen content (as ammonia NH_3–N) with the use of the phenate method (American Public Health Association et al. 2012). Nitrogen values were compared to atLeaf+ predicted chlorophyll values by Pearson product moment correlation and regression analysis with the use of Statistica version 12 (StatSoft 2013).

RESULTS AND DISCUSSION

A significant ($P < 0.05$, $r = 0.839$) positive correlation was detected between predicted chlorophyll values and actual nitrogen levels in water hyacinth above-water biomass (Figure 1). Greater than 70% of the variation in mean nitrogen levels can be explained by the linear relationship between estimated chlorophyll and actual plant nitrogen content. These correlations are similar to those reported with the use of the SPAD-520 meter to determine nitrogen levels in water hyacinth leaves (Dray et al. 2012). However, the amount of variation explained by the atLeaf+ meter measurements is larger in comparison to those for the SPAD-520 meter (coefficient of determination: 70% vs 53%, respectively). The smaller coefficient of determination for the correlation demonstrated by Dray et al. (2012) may be caused by combining data from two different experiments examining different fertilization levels and presence of herbivory to detail the relationship between the SPAD-520 meter readings and actual nitrogen content.

The largest source of unaccounted variation between predicted chlorophyll and nitrogen is believed to be related to the plant parts used in the determination of nitrogen in this experiment. Nitrogen was measured on the entire above-water biomass (including leaves and petioles), in contrast to the atLeaf+ measurements, which were taken only on leaf tissue. Water hyacinth petioles generally contain significantly lower chlorophyll and nitrogen content in comparison to leaf tissues (Dray et al. 2012). To develop more precise relationships and limit the observed variation, nitrogen should be measured on the same leaf tissues that were used to determine chlorophyll levels using the atLeaf+ meter. It has also been reported that there is an upper limit in which the atLeaf+ meter will measure chlorophyll adequately; very high levels of chlorophyll, as an indicator of nitrogen content, result in curves that eventually reach an asymptotic plateau and can thus lead to erroneous estimation of nitrogen (Coste et al. 2010). We did not observe such a phenomenon, but plants containing very high levels of luxury nitrogen (i.e., the increased storage of N without an associated influence on biomass production; Lipson et al. 1996) may not be accurately measured with the use of the atLeaf+ meter alone. Other sources of variation in predicting nitrogen, based solely on the use of light transmittance chlorophyll meters, include leaf thickness, leaf position, interactions with mineral content, and differences in leaf mass per area (Coste et al. 2010, Loh et al. 2012). To minimize such variation, an average based on multiple readings from each leaf and/or multiple leaves is recommended. Similar recommendations were given by Dray et al. (2012).

Although additional experiments are warranted to elucidate the relationship between chlorophyll readings as measured by the atLeaf+ meter and nitrogen content in water hyacinth leaves, there is adequate evidence to support the use of the atLeaf+ meter to estimate nitrogen in water hyacinth leaf tissues. The use of this technique provides a nondestructive method that is easy, rapid, and less expensive than traditional laboratory-based tissue analyses or more expensive chlorophyll meters. Tissue nitrogen content is an important indicator of plant health, and the susceptibility of plants to disease and pest attack (Miladinovic et al. 2008) and the ability to determine it nondestructively opens up a variety of uses, including in situ assessment of plant quality with respect to biocontrol agent field establishment and performance, experimental studies designed to evaluate plant nutritional quality under different fertilization regimes, and the overall health of plants used in aquatic plant restoration programs. Although chlorophyll meters have not been used to determine aquatic plant health and potential need for additional fertilization in restoration project cultures, their utility in this respect has been documented for multiple crop species (Schepers et al. 1992, Shapiro et al. 2013). In addition, nitrogen has been shown to influence reproductive capacity strongly in two biocontrol agents (i.e., *Neochetina eichhorniae* and *Neochetina bruchi*) released for the management of water hyacinth (Center and Dray 2010, Center et al. 2014) so understanding nitrogen content in field plants may provide a more complete understanding of establishment and impact success of these agents. However, the measured and predicted nitrogen values presented herein show that the relationship between atLeaf+ readings and actual nitrogen content is not exact; a large amount of variation is not fully explained, so caution should be used when presenting estimated nitrogen, emphasizing that values were determined via predictive equations only. Also, when applying this technique to other aquatic plant species it is important to develop standardized fits for each species under consideration. It has been shown that different relationships and fits are often species specific (Coste et al. 2010).

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