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Nondestructive Estimation of Leaf Area for Pondberry

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Abstract

Pondberry (*Lindera melissifolia* [Walt.] Blume) is a federally listed endangered shrub found as isolated populations in seasonally flooded forests across the Southeastern United States. Because this shrub is rare, it has received little research attention, and basic knowledge of its ecology and physiology is lacking. To facilitate future ecological and physiological studies on pondberry, we developed and tested a model to predict area of individual leaf blades from simple dimensions that are obtained nondestructively. A linear function, using the product of blade length and width as the independent variable, was found to be the most suitable predictor of pondberry leaf blade area based on correlation coefficients ($r^2 = 0.9956$), plots of actual versus predicted values, and predicted versus residual values. We demonstrate that simple dimensions that are obtained nondestructively, such as blade length and width, can be used to reliably predict leaf blade area of pondberry, but model coefficients should be calibrated for local colonies to improve estimates. Development of this model allows for leaf blade area determination at the plant level without the need to destructively harvest foliage.

Introduction

Pondberry (*Lindera melissifolia* [Walt.] Blume), also known as hairy spicebush, southern spicebush, or swamp spicebush, is a member of the Lauraceae. This rhizomatous shrub grows up to 2 m tall in seasonally flooded forests across the Southeastern United States (Devall and others 2001, Radford and others 1968, U.S. Fish and Wildlife Service 1993). Pondberry has been described as one of the rarest shrubs in the United States (Steyermark 1949), and is a federally listed endangered species (U.S. Fish and Wildlife Service 1986). A subsequent recovery plan published in 1993 (U.S. Fish and Wildlife Service 1993) documents the need to acquire additional knowledge about the biology and ecology of this species. The lack of biological and ecological knowledge of pondberry has hampered development of strategies for recovery of pondberry and removal of the plant from the endangered species list.

A key to increasing knowledge of pondberry growth and development is data on cause-and-effect relationships between species physiology and various environmental factors, such as light availability, soil moisture availability, and response to competition. Ledig (1976) stated that information about the partitioning of growth among plant tissues is necessary to understand how environmental conditions and cultural practices affect growth. Leaf area is widely considered an important index of plant growth and development (Larsen and Kershaw 1991, Ramkhelawan and Brathwaite 1990) because it is directly related to light interception, carbon assimilation, and gas exchange (Lu and others 2004). Methods for determining leaf area vary, but a common technique involves building regression equations that predict leaf blade area from measurements of blade or stem variables, e.g., blade length, blade width, petiole length, and internode length (Larsen and Kershaw 1991). These area models can then be used to compute total leaf area of a plant without destructively sampling leaves (Bange and others 2000, Guo and others 1995). Since the availability of a nondestructive technique for estimating leaf area would advance future research on the species, the objective of this study was to develop a model to predict pondberry leaf blade area based on simple, nondestructive measurements of leaf or stem dimensions.

Materials and Methods

Pondberry leaf material used in this experiment was harvested from 28 seedlings raised in a growth chamber.

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These seedlings were established from seed collected on the Delta National Forest (latitude 32° 58′ N., longitude 90° 44′ W.), near Rolling Fork, MS. In May 2004, seed that had been stratified for 3 months at 2 °C was potted in 7.5-L containers with a soil mix consisting of peat moss, perlite, 0–46–0 and 10–10–10 (nitrogen–phosphorus–potassium) fertilizer, and Milorganite (manufactured by Milwaukee Metropolitan Sewage District, Milwaukee, WI). Seed were germinated and seedlings were grown for 3 months under one of two environmental conditions—high-light availability or low-light availability. These treatment levels were selected to produce a range in pondberry leaf sizes for development of a robust model for leaf blade area prediction. The 14 seedlings receiving high light were exposed to a maximum of 600 μmol m^2 per second photosynthetically active radiation (PAR) while those receiving low light were exposed to a maximum of 120 μmol m^2 per second PAR. The low-light regime was implemented in the growth chamber by placing 63 percent neutral density shade cloth above the pots assigned to this treatment. Both light regimes were applied for 16 hours each day and included a daily 2-hour step up and 2-hour step down period during which light levels were increased or decreased by one quarter each half hour. Daytime temperature was maintained at 26 °C, nighttime temperature was maintained at 20 °C, and all seedlings were watered three to four times per week to maintain adequate soil moisture.

Following 3 months of growth, all leaves were excised from each seedling. Total leaf blade length (mm) along the midrib, width (mm) at the widest point perpendicular to the midrib, and the subtending internode length (mm) were measured with a ruler. Area (cm^2) of each leaf blade was measured twice with a LiCor LI3100 leaf area meter and the two measurements for each leaf blade were averaged. We evaluated the suitability of eight linear regression models developed to predict blade area. Models included various combinations of blade length, width, and subtending internode length used as independent variables, and the averaged blade area used as the dependent variable. We also tested for differences between leaves grown under high and low light availability. These tests for differences in leaf blade length, width, length/width ratio, and leaf blade area were conducted with *t*-tests at an alpha level of 0.05.

To further assess the usefulness of the selected model to predict leaf area of wild plants, we sampled an additional 90 leaves (30 from each of 3 wild pondberry colonies) growing at a field site about 10 km north of Cleveland, MS (latitude 32° 45′ N., longitude 88° 50′ W.). Dimensional measurements and dimensions of leaf blade area were conducted on these leaves as described above. Regression analyses with dummy variables were used to compare coefficients of leaf blade area models developed for plants grown in the growth chamber with those of plants grown in the field. All data analyses and modeling were conducted with PC–SAS (SAS 1985).

Results and Discussion

Pondberry leaves are simple, have a smooth margin, and are generally oblong with a pointed apex and a round base. Table 1 presents basic leaf characteristics measured on pondberry seedlings examined in this study. Leaf blade length, width, and area of plants studied in this research differed according to light regime. Leaves from seedlings grown under low light developed blades 35 percent longer and 40 percent wider than leaves of seedlings grown under high light (table 1). Shade leaves are commonly larger than sun leaves (Gardiner and others 2004, Givnish 1988, Hamerlynck and Knapp 1994, Lichtenthaler and others 1981). Also, blade area of leaves that developed under low light was 64 percent greater than that of leaves that developed under high light. Greater pondberry total leaf area under low- and intermediate-light regimes has been

Table 1—Leaf blade characteristics of pondberry seedlings grown under high and low light regimes in a growth chamber

	n	Length	Width	Ratio (length/width)	Blade area
		- - - - - - *mm* - - - - - -			*cm*^{2}
High light[a]	320	57 ± 2[b]	24 ± 1	2.32 ± 0.02	10.8 ± 0.6
Range[c]		11 – 130	5 – 53	1.20 – 3.40	0.1 – 40.8
Low light	471	88 ± 2	40 ± 1	2.28 ± 0.02	30.0 ± 1.3
Range		11 – 184	5 – 106	1.21 – 3.60	0.1 – 136.0
p-value		0.0001	0.0001	0.1516	0.0001

[a] Seedlings under the high light regime received up to 600 μmol m^2 per second photosynthetically active radiation (PAR), while those under the low light regime received up to 120 μmol m^2 per second PAR.
[b] Mean ± 1 standard error of the mean.
[c] Range is minimum and maximum values.

reported previously (Aleric and Kirkman 2005). In our study, the ratio of leaf blade length to leaf blade width was the same for plants that were grown under high and low light conditions. This finding meant that the high light leaves and the low light leaves had the same shape and allowed us to pool measurements from both treatments for the regression analyses discussed below.

The eight linear regression models tested for leaf blade area prediction differed in effectiveness (table 2). Plots of leaf blade length and width indicated that simple linear regression without data transformation would provide adequate prediction of pondberry leaf blade area. Internode length was not as strong a predictor of blade area as were blade length and width (table 2). Equation 3 was selected as the most suitable predictor of pondberry leaf blade area based on ease of measurement, time to conduct measurements (length and width versus length or width), high correlation coefficients, the plot of actual versus predicted values (fig. 1), and the plot of predicted versus residual values

Table 2—Linear equations tested to predict leaf blade area of pondberry raised under two light regimes in a growth chamber

Equation number	Model[a]	r^2	Mean square error	Standard error of regression coeff.
1	A = – 19.0700 + 0.5413 (length)[b]	0.8588	9.29	0.0078
2	A = – 17.2138 + 1.1558 (width)	0.9380	6.16	0.0106
3	A = – 0.88340 + 0.0067 (length x width)	0.9956	1.64	0.0007
4	A = – 15.0245 + 2.1915 (internode)	0.5726	16.16	0.0674
5	A = – 2.0004 + 0.0032 (length x length)	0.9475	5.66	0.00003
6	A = 1.617472 + 0.012850 (width x width)	0.9695	4.32	0.0001
7	A = – 17.351268 + 0.014248 (length)	0.9381	6.16	0.0174
	+ 1.128022 (width)			0.0356
8	A = – 0.754028 + 0.001414 (length x length)	0.9966	1.45	0.00002
	+ 0.007617 (width x width)			0.00007

[a] *P*-value for regression was 0.0001 for all equations.
[b] A = predicted leaf blade area in cm^2; length = leaf blade length (mm) along the midrib; width = leaf blade width (mm) at the widest point perpendicular to the midrib; internode = subtending internode length in mm.

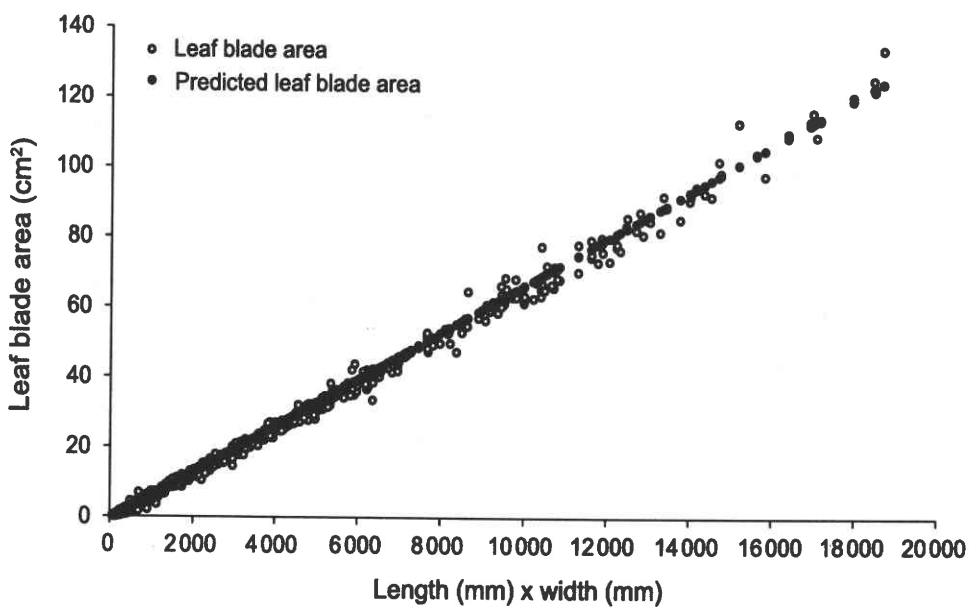

Figure 1—Leaf blade area versus predicted leaf blade area of pondberry using blade length times blade width as the independent variable.

(fig. 2). The estimated gain (r^2) of the product of blade length and width versus length was 0.14 and that of the product of blade length and width versus width was 0.06. This improvement justifies the additional time necessary to measure both leaf blade length and width rather than measuring only a single dimension. The 0.001 gain observed for Equation 8 was not considered high enough to warrant use of multiple linear regression to predict pondberry leaf blade area.

The cross product of leaf length and width has been highly correlated with leaf blade area in several other plant species. Applications of such models to broadleaf tree species are discussed in Guo and others (1995) for cherrybark oak (*Quercus pagoda* Raf., $r^2 = 0.98$) and in Kubicek (1971) for sessile oak (*Q. petraea* [Mattuschka] Liebl., $r^2 = 0.97$). Applications to herbaceous dicots are discussed in Bange and others (2000) for sunflower (*Helianthus annuus* L., $r^2 = 0.94$) and in Hughes and others (1979) for pigeon pea (*Cajanus cajan* [L.] Millsp., where r^2 ranged from 0.91 to 0.98 depending on cultivar). Chanda and others (1985) and Krishnamurthy and others (1974) presented examples with monocots including pearl millet (*Pennisetum americanum* [L.] Leeke, $r^2 = 0.998$) and grain sorghum (*Sorghum bicolor* [L.] Moench, where r^2 ranged from 0.956 to 0.987 depending on variety). Thus, models of this type have been used to predict leaf blade area over a range of plant types.

As described in the Materials and Methods section, we collected pondberry leaves from a nearby field site and used them to test the capability of the model to predict leaf blade area of wild plants. Leaves harvested in the field were generally larger in length, width, and area than those grown in the growth chamber (table 3). The slope coefficient for

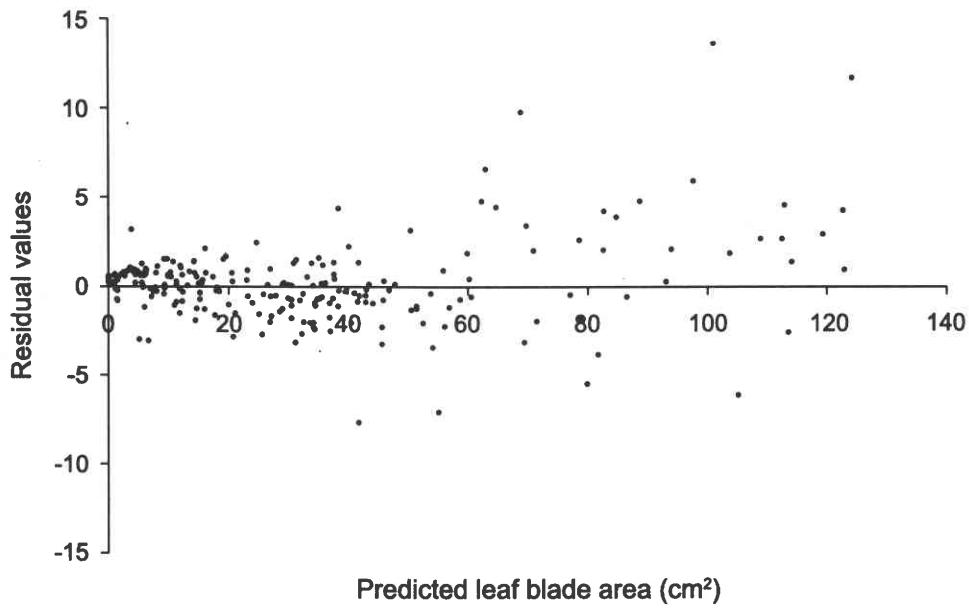

Figure 2—Plot of residual versus predicted leaf blade area for pondberry raised under two light regimes in a growth chamber.

Table 3—Characteristics of leaf blades harvested from wild pondberry colonies, Boliver County, MS

Colony	Gender	n	Length	Width	Ratio (length/width)	Blade area
			------ *mm* ------			*cm²*
1	Male	30	102 ± 6[a]a	48 ± 3 a	2.17 ± 0.03 a	35.3 ± 3.9 ab
2	Male	30	120 ± 7 a	52 ± 4 a	2.36 ± 0.05 b	48.3 ± 5.7 a
3	Female	30	100 ± 7 a	43 ± 3 a	2.37 ± 0.06 b	34.0 ± 4.5 b
p-value			0.0756	0.1719	0.0059	0.0706

[a] Mean ± 1 standard error of the mean.
Means within a column followed by different letters are significantly different ($p \leq 0.05$).

growth chamber plants differed from that for field-grown plants ($p < 0.0001$). Two of the three wild colonies had slope coefficients different from that for growth chamber plants ($p = 0.5435$, 0.0232, and 0.0041 for colonies 1, 2, and 3, respectively). These results indicate that calibrating the model for pondberry colonies of interest may improve prediction of leaf blade area for field-grown plants.

In summary, we developed a simple, nondestructive method for predicting leaf blade area of pondberry from basic leaf dimensions. A linear function with the product of blade length and width as the independent variable was found to be the most suitable predictor of pondberry leaf blade area (table 2). This function and easily obtained leaf measurements can be used to accurately estimate pondberry leaf blade area, though the function may need to be calibrated for local colonies. The availability of this function will enable researchers to develop comprehensive ecological and physiological studies that involve leaf area dynamics over multiple scales. Such research will lead to a more complete understanding of the environmental requirements for pondberry growth and development, which is critical to the development and implementation of management strategies that will hasten the recovery of this endangered species.

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Literature Cited

Aleric, K.M.; Kirkman, L.K. 2005. Growth and photosynthetic responses of the federally endangered shrub, *Lindera melissifolia* (Lauraceae), to varied light environments. American Journal of Botany. 92: 682-689.

Bange, M.P.; Hammer, G.L.; Milroy, S.P.; Rickert, K.G. 2000. Improving estimates of individual leaf area of sunflower. Agronomy Journal. 92: 761-765.

Chanda, S.V.; Joshi, A.K.; Vaishnav, P.P.; Singh, Y.D. 1985. Leaf area determination in pearl millet using linear measurements-area and matter-area relationships. Photosynthetica. 19: 424-427.

Devall, M.; Schiff, N.; Boyette, D. 2001. Ecology and reproductive biology of the endangered pondberry, *Lindera melissifolia* (Walt) Blume. Natural Areas Journal. 21: 250-258.

Gardiner, E.S.; Stanturf, J.A.; Schweitzer, C.J. 2004. An afforestation system for restoring bottomland hardwood forests: biomass accumulation of Nuttall oak seedlings interplanted beneath eastern cottonwood. Restoration Ecology. 12: 525-532.

Givnish, T.J. 1988. Adaptation to sun and shade: a whole plant perspective. Australian Journal of Plant Physiology. 15: 63-92.

Guo, Y.; Lockhart, B.; Hodges, J. 1995. Prediction of leaf area in individual leaves of cherrybark oak seedlings (*Quercus pagoda* Raf.). Proceedings of the Arkansas Academy of Science. 49: 52-55.

Hamerlynck, E.P.; Knapp, A.K. 1994. Leaf-level responses to light and temperature in two co-occurring *Quercus* (Fagaceae) species: implications for tree distribution patterns. Forest Ecology and Management. 68: 149-159.

Hughes, G.; Keatinge, J.D.H.; Scott, S.P. 1979. Leaf area estimation by non-destructive methods in pigeon pea (*Cajanus cajan* (L.) Millsp.). Tropical Agriculture. 56: 371-374.

Krishnamurthy, K.; Jagannath, M.K.; Rajashekara, B.G.; Raghunatha, G. 1974. Estimation of leaf area in grain sorghum from single leaf measurements. Agronomy Journal. 66: 544-545.

Kubicek, F. 1971. Leaf area estimation in *Quercus petraea* using linear regression equations. Photosynthetica. 5: 426-428.

Larsen, D.R.; Kershaw, J.A., Jr. 1991. Leaf area measurements. In: Lassoie, J.P.; Hickley, T.M., eds. Techniques and approaches in forest tree ecophysiology. Boca Raton, FL: CRC Press: 465-475.

Ledig, F.T. 1976. Physiological genetics, photosynthesis and growth models. In: Cannell, M.G.R.; Last, F.T., eds. Tree physiology and yield improvement. New York: Academic Press: 21-54.

Lichtenthaler, H.K.; Buschmann, C.; Doll, M. [and others]. 1981. Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosynthesis Research. 2: 115-141.

Lu, H.; Lu, C.; Wei, M.; Chan, L. 2004. Comparison of different models for nondestructive leaf area estimation in taro. Agronomy Journal. 96: 448-453.

Radford, A.E.; Ahles, H.E.; Bell, C.R. 1968. Manual of the vascular flora of the Carolinas. Chapel Hill, NC: University of North Carolina.

Ramkhelawan, E.; Brathwaite, R.A.I. 1990. Leaf area estimation by non-destructive methods in sour orange (*Citrus aurantium* L.). Tropical Agriculture. 67: 203-206.

SAS Institute Inc. 1985. SAS/STAT guide for personal computers. Version 6. edition. Cary, NC: SAS Institute Inc.

Steyermark, J.A. 1949. *Lindera melissaefolia*. Rhodora. 51: 153-162.

U.S. Fish and Wildlife Service. 1986. Endangered and threatened wildlife and plants: determination of endangered status for *Lindera melissifolia*. Federal Register. 51: 27,495-27,499.

U.S. Fish and Wildlife Service. 1993. Recovery plan for pondberry (*Lindera melissifolia*). Atlanta.

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