Variation in Leaf Nutrient Concentrations of Freeman Maple Resulting from Canopy Position, Leaf Age, and Petiole Inclusion

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Abstract. Consistent evaluation of nursery crop nutrient status within and among plots and years requires careful consideration of leaf collection practices. The objectives of this study were to determine the differences and variability among leaf age and cardinal position within the tree. Another objective was to determine if petioles should be included in leaf samples. Leaves were collected from seven trees of the Freeman maple (red–silver maple hybrid) Celebration® (Acer x freemanii) from two leaf ages (early- and late-season leaves) and four positions (north, south, east, and west). On the south side of each tree, samples were separated into lamina, petiole, or lamina + petiole samples. Leaf traits were not different among positions, except that leaves on the north side of the tree had a lower specific leaf weight (SLW). Leaf nitrogen (N) was slightly lower on the north and west sides of the tree and leaf calcium (Ca) was highest on the south side of the tree, but otherwise position (i.e., side of the tree) had very little effect on leaf nutrient concentrations. Older leaves (leaves produced early in the season) were darker green and had a higher leaf area, longer petiole length, and lower SLW. Older leaves also had higher concentrations (on a mass basis) of all nutrients analyzed. Petiole concentrations of Ca, magnesium, and manganese were higher than lamina concentrations, whereas concentrations of N, sulfur, iron, zinc, boron, copper, and aluminum were all higher in the lamina. These differences, however, did not affect nutrient analyses conducted on samples consisting of lamina + petiole or lamina only. Variation within samples was lowest on the east and south sides for macro- and micronutrients, respectively, in late-season leaves. Based on the relative variation within samples, samples for nutrient analysis of red maple and red maple hybrids should consist of leaves collected from the southeast side of the tree and can consist of samples with or without petioles attached to the lamina.

Leaf nutrient status is an important tool for growers in determining fertilizer needs in ornamental species. This is especially true as consciousness and regulation of fertilizer runoff and leaching into groundwater increases. However, growers often do not have the resources to test a large number of plants or portions of plants. It is therefore crucial that sampling procedures are designed to collect the most representative samples within a plot or nursery. Furthermore, because nutrient concentration of leaves can vary greatly as a result of position in the canopy, sample collection should be designed to obtain the most uniform and representative samples possible.

Freeman maple is a popular landscape (Iles and Vold, 2003) and street tree (Snyder and Struve, 1997). The goal of this study was to determine the differences in leaf characteristics and nutrient concentrations resulting from position (north, south, east, and west directions) and age (early-season versus late-season leaves). Furthermore, nutrient concentrations of lamina and petiole were determined to provide information for proper sampling techniques.

Materials and Methods

Field characteristics. Leaves were collected from Acer x freemanii Celebration® trees that are part of a larger plot at the Purdue Meigs Horticulture Farm located in Tippecanoe County, IN (lat. 40.288, long. 86.883). The trees were planted in 2007 as 1-year-old cutting-propagated-whips. Soil was sampled with an auger to 15 cm deep at the four cardinal positions (north, west, south, east) at the edge of the canopy of each tree used in the study. Soil samples from each position were combined for each tree and submitted for analysis. Mean values for soil characteristics are presented in Table 1.

Plant characteristics. Tree height was measured from soil level to the highest growing point. Branch length of each branch used for leaf collection was also measured from its origin at the trunk to the farthest growing point. Canopy volume was calculated from average measurements of canopy width in four cardinal directions and the distance from the bottom of the canopy to the top. The canopy was assumed to be a cone and canopy volume was calculated as $V = (1/3)\pi r^2 h$. Trunk diameter at 15 cm above soil level was measured with digital calipers in both the north–south and east–west directions. The average radius of these measurements was used to calculate trunk cross-sectional area as $TCA = \pi r^2$, where $r$ is the radius of the average trunk caliper.

Leaf collection and analysis. Leaves were collected in Aug. 2009 from individual shoots in four quadrants of the canopy: north, west, south, and east. Within each quadrant, leaves were collected from the outer third (late season leaves) and the inside third (early season leaves) of a given shoot. Early-season leaves were produced at budbreak (approximately April), and late-season leaves were produced approximately early June.

For analysis of petiole and leaf characteristics, a separate pool of 20 leaves was collected from the south quadrant of the tree. The 20 leaves were then randomly distributed into two pools of 10 leaves. One pool of 10 leaves was analyzed for nutrient concentrations: 1) whole leaf (lamina + petiole); 2) lamina only; and 3) petiole only (from the same leaf used for Sample 2).

Petiole length and leaf area (LA) were calculated from digital photographs using open-source, public domain ImageJ software (http://rsbweb.nih.gov/ij/index.html). Leaf area to dry weight ratio was determined from 20 leaves from each of four positions for both early- and late-season leaves. The ratio was different between leaf ages, but not among positions, so the pooled data were used to generate a standard curve for each leaf age estimating leaf dry weight from area for all collected samples. Specific leaf weight (SLW) was determined in each harvested leaf by measuring the disc dry weight (DDW) of a 1.33-cm² disc [disc leaf area (DLA)] removed from the lamina with a cork borer and calculating $SLW = DDW/DLA$. Leaf greenness was determined using a SPAD 502 chlorophyll meter (Konica Minolta, Tokyo, Japan).

Leaves for nutrient analysis were dried to a constant dry weight at 80 °C in a forced-air oven and ground to a fine powder using a coffee grinder. Nitrogen was analyzed using the Dumas combustion procedure (Simonne et al., 1994). All other elements were digested with 1.0 mL of HNO₃ at 110 °C for 4 h and quantified by inductively coupled plasma spectrometry on a PerkinElmer Elan DRCe ICP-MS (PerkinElmer SCIX, Shelton, CT).

Experimental design and statistical analysis. The data were analyzed as a two-way factorial (position and leaf age) on a split plot with seven trees representing blocks. Canopy position was the main plot and leaf age (branch position within a direction) was the subplot. In all analyses, the position $\times$ leaf
Table 1. Range of soil characteristics (top 15 cm) under the canopy of trees from which samples were obtained for determination of leaf nutrient concentrations in leaves of different ages and positions in the canopy (n = 7).a

| Variable               | Unit     | Value     |
|------------------------|----------|-----------|
| pH                     |          | 7.3 ± 0.2 |
| Buffer pH              |          | 7.2 ± 0.0 |
| Organic matter (%)     |          | 1.9 ± 0.2 |
| Cation exchange capacity meq/100 g |          | 10.8 ± 0.6 |
| NO₃⁻N                  | mg·kg⁻¹ | 12.1 ± 0.2 |
| NH₄⁺N                 | mg·kg⁻¹ | 3.5 ± 0.5 |
| Phosphorus             | mg·kg⁻¹ | 11.7 ± 1.6 |
| Potassium              | mg·kg⁻¹ | 13.1 ± 3.6 |
| Calcium                | mg·kg⁻¹ | 1621.4 ± 80.9 |
| Magnesium              | mg·kg⁻¹ | 290.0 ± 21.2 |
| Sulfur                 | mg·kg⁻¹ | 7.9 ± 1.6 |
| Zinc                   | mg·kg⁻¹ | 2.0 ± 0.0 |
| Manganese              | mg·kg⁻¹ | 55.4 ± 4.2 |
| Iron                   | mg·kg⁻¹ | 16.0 ± 4.4 |
| Copper                 | mg·kg⁻¹ | 1.5 ± 0.1 |
| Boron                  | mg·kg⁻¹ | 0.6 ± 0.2 |

Table 2. Leaf greenness (SPAD), leaf area (LA), petiole length (PL), and specific leaf weight (SLW) of leaves collected from four positions (north, west, south, or east) or from late- or early-season leaves of ‘Celebration’ Freeman maple.a

| Position | LA (cm²) | PL (cm) | SLW (g·cm⁻²) |
|----------|----------|---------|--------------|
| North    | 40.3     | 48.2    | 6.85         |
| West     | 38.5     | 49.6    | 7.03         |
| South    | 39.6     | 47.5    | 6.97         |
| East     | 39.7     | 48.3    | 7.14         |

Significance a

Table 3. Mean nutrient concentrations of leaves collected from four positions (north, west, south, or east) or from late- or early-season leaves (n = 7) of ‘Celebration’ Freeman maple.a

| Position | Nitrogen (mg·g⁻¹) | Phosphorus (mg·g⁻¹) | Potassium (mg·g⁻¹) | Sulfur (mg·g⁻¹) | Calcium (mg·g⁻¹) | Magnesium (mg·g⁻¹) | Iron (mg·g⁻¹) | Manganese (mg·g⁻¹) | Zinc (mg·g⁻¹) | Boron (mg·g⁻¹) | Copper (mg·g⁻¹) | Aluminum (µg·g⁻¹) |
|----------|------------------|---------------------|--------------------|-----------------|-----------------|------------------|--------------|--------------------|--------------|---------------|----------------|------------------|
| North    | 2.42 ab          | 0.181               | 0.68               | 0.18            | 2.41 b          | 0.45             | 89.4         | 58.0               | 20.1         | 50.4          | 5.43           | 22.5             |
| West     | 2.37 b           | 0.174               | 0.68               | 0.18            | 2.41 b          | 0.45             | 88.6         | 64.7               | 21.7         | 50.5          | 5.43           | 23.8             |
| South    | 2.50 a           | 0.188               | 0.66               | 0.19            | 2.69 a          | 0.48             | 97.6         | 67.9               | 21.1         | 51.0          | 5.21           | 25.4             |
| East     | 2.47 a           | 0.185               | 0.65               | 0.19            | 2.46 b          | 0.45             | 98.5         | 60.6               | 20.4         | 49.2          | 5.21           | 19.9             |

Significance a

Table 3. Mean nutrient concentrations of leaves collected from four positions (north, west, south, or east) or from late- or early-season leaves (n = 7) of ‘Celebration’ Freeman maple.a

| Position | Nitrogen (mg·g⁻¹) | Phosphorus (mg·g⁻¹) | Potassium (mg·g⁻¹) | Sulfur (mg·g⁻¹) | Calcium (mg·g⁻¹) | Magnesium (mg·g⁻¹) | Iron (mg·g⁻¹) | Manganese (mg·g⁻¹) | Zinc (mg·g⁻¹) | Boron (mg·g⁻¹) | Copper (mg·g⁻¹) | Aluminum (µg·g⁻¹) |
|----------|------------------|---------------------|--------------------|-----------------|-----------------|------------------|--------------|--------------------|--------------|---------------|----------------|------------------|
| Late     | 2.24             | 0.15                | 0.59               | 0.17            | 1.55            | 0.34             | 91.7         | 40.8               | 19.9         | 41.5          | 4.46           | 15.0             |
| Early    | 2.65             | 0.21                | 0.75               | 0.20            | 3.43            | 0.58             | 95.5         | 84.2               | 21.8         | 59.0          | 5.79           | 30.8             |

Significance a
older leaves (Jabine and Tomar, 2008). It appears that leaf nutrient concentrations in leaves of different ages vary both among and within plant types (i.e., deciduous versus evergreen).

Nutrient concentration differences between the lamina and petiole within leaves can potentially skew results and recommendations if samples are collected differently among growers or years. The degree to which the petiole nutrient concentrations affect leaf analysis values depends on the proportion of the total leaf weight that the petiole represents and how that proportion changes within the canopy. Leaf and petiole dry weight and the proportion of the dry weight accounted for by the petiole did not differ with position within the canopy (Table 4). Petiole weight of early-season leaves was similar to late-season leaves (Table 4), but early-season petioles were longer (Table 2). Furthermore, early-season leaves were smaller with lower SLW (Table 2) and were therefore approximately half the weight of late-season leaves. This resulted in the petiole accounting for ≈22% of the total leaf dry weight in early-season leaves, whereas it only accounted for ≈12% of the total dry weight in late-season leaves.

Leaf lamina had higher concentrations of N, sulfur, Fe, Zn, B, copper (Cu), and Al than petioles (Table 5). Conversely, petioles had two times the concentration of Ca and 1.8 and 1.9 times the concentrations of Mg and Mn, respectively. These differences in nutrient concentration would be important considerations in developing sampling protocols if the analysis values obtained differ in samples with and without petioles. However, in no cases were concentrations different in analyses done on leaves with and without petioles. This was also true when a two-way analysis was done between lamina and petiole + petiole samples (i.e., removing petiole-only samples from the analysis; results not shown). Although the concentrations of Ca, Mg, and Mn were approximately twofold higher in petioles than in the lamina (Table 5), because the petiole accounts for only slightly over 10% of the total leaf weight (Table 4), the analysis results are unaffected by the presence or absence of the petiole in this Freeman maple and is also likely the case in other red and red × silver maple hybrids with similar lamina:petiole ratios. Likewise in kiwifruit (Actinidia delicosa Chev.), although lamina and petiole nutrient concentrations were different, the inclusion of the petiole in the leaf sample (i.e., leaf + petiole) did not appear to affect the overall tissue analysis results (Sharma et al., 2005). This may not be true for other species in which the petiole constitutes a higher percentage of the total leaf weight (e.g., honeylocust).

In several wine grape (Vitis vinifera L.) cultivars, K, Mg, and Zn were higher in petioles, whereas Ca, Fe, and Mn were lower (Fallahi et al., 2005). Petioles had lower Ca, Mg, and Mn but higher K in the herbaceous plant taro (Colocasia esculenta L.) (Hill et al., 1998). All of these trends except Fe are contrary to our results. Sharma et al. (2005) found that petioles had higher concentrations of Ca and Mg than leaf blades in kiwifruit, which corresponds with our data. However, they also showed that P and K concentrations were higher in petioles and that Cu and Zn were higher in petiole in kiwifruit. These trends in kiwifruit were also observed in grape (Cummings, 1977). The relative nutrient concentrations observed in this study were contrary to several other published reports, suggesting that maple may be unique among woody species or that variability in these ratios is high among species. It is also conceivable that cultivars may differ in these relative concentration differences and therefore analyses may differ with collection method among cultivars. Differences in petiole nutrient concentrations have also been shown to differ as a result of rootstock in grape (Wolpert et al., 1995).

An important consideration when sampling leaf tissue for nutrient analysis is the inherent variability in the tissue. This is especially true for growers who are often sampling from few trees or have a low total number of samples to evaluate. Based on the CV for samples collected, the lowest variability in nutrient concentrations for late-season leaves tended to be on the east (macronutrients)

Table 4. Lamina (LDW) and petiole (PDW) dry weight and petiole percent of total lamina (%P) of ‘Celebration’ Freeman maple leaves in different positions (north, west, south, and east) and of different ages.1

| Position | LDW (mg) | PDW (mg) | %P (%) |
|----------|----------|----------|--------|
| North    | 429      | 72       | 16.6   |
| West     | 435      | 76       | 16.7   |
| South    | 414      | 77       | 17.2   |
| East     | 423      | 79       | 17.2   |
| Significance2 | NS      | NS      | NS     |

1Late leaves are those that were produced in June and were located in the terminal third of a main shoot. Early leaves are those that were produced in April and were located in the basal third of a main shoot.

2Differences among means within a column are nonsignificant (NS) or significant at *P ≤ 0.05, **0.01, or ***0.001 based on Tukey’s least significant difference test.

Table 5. Mean leaf nutrient concentrations of lamina (L), petioles (P), and lamina + petiole (LP) collected from the south position from late-season leaves (n = 7) of ‘Celebration’ red × silver maple.

| Leaf part | Nitrogen | Phosphorus | Potassium | Sulfur | Calcium | Magnesium | Iron | Manganese | Zinc | Copper | Aluminum |
|-----------|----------|------------|-----------|--------|---------|-----------|------|------------|------|---------|-----------|
| LP        | 2.28 a   | 0.16       | 0.57      | 0.18 a | 1.80 b  | 0.37 b    | 94.3 a| 45.1 b     | 20.4 a| 42.1 a  | 4.43 a    |
| L         | 2.37 a   | 0.16       | 0.58      | 0.18 a | 1.47 b  | 0.33 b    | 106.1 a| 39.4 b     | 20.0 a| 42.6 a  | 4.14 a    |
| P         | 1.29 b   | 0.15       | 0.62      | 0.12 b | 3.10 a  | 0.61 a    | 26.4 b| 75.9 a     | 39.4 b| 22.4 a  | 6.29 b    |
| Significance2 | ***      | NS        | NS        | **     | ***     | ***       | *    | ***        | **    | ***     | ***       |

1Differences among means within a column are nonsignificant (ns) or significant at *P ≤ 0.05, **0.01, or ***0.001 based on Tukey’s least significant difference test.

Table 6. cv for leaf nutrient concentrations of leaves collected from four positions (north, west, south, or east) in both late- (LL) or early- (EL) season leaves and from late season lamina + petiole (LP), lamina only (L), or petiole only (P) (n = 7) of ‘Celebration’ red × silver maple.1

| Tissue  | Position | Nitrogen | Phosphorus | Potassium | Sulfur | Calcium | Magnesium | Iron | Manganese | Zinc | Copper | Aluminum |
|---------|----------|----------|------------|-----------|--------|---------|-----------|------|------------|------|---------|-----------|
| LL      | North    | 5.18     | 5.98       | 7.96      | 3.01   | 13.21   | 8.03      | 11.47| 32.98      | 23.23| 17.21   | 38.91     |
| LL      | West     | 5.26     | 10.79      | 14.60     | 9.21   | 13.53   | 8.83      | 12.95| 20.64      | 16.44| 16.66   | 44.38     |
| LL      | South    | 6.81     | 11.61      | 11.24     | 8.79   | 23.56   | 15.15     | 21.30| 16.70      | 14.78| 12.07   | 54.48     |
| LL      | East     | 3.76     | 4.44       | 5.99      | 4.55   | 8.16    | 6.34      | 19.54| 27.66      | 14.66| 8.83   | 16.03     |
| EL      | North    | 3.71     | 8.96       | 13.51     | 2.80   | 7.38    | 6.21      | 10.84| 17.09      | 9.60 | 9.48   | 23.80     |
| EL      | West     | 3.53     | 6.23       | 12.87     | 5.75   | 2.25    | 3.25      | 12.37| 24.69      | 7.94 | 10.35  | 7.35      |
| EL      | South    | 2.88     | 9.74       | 12.11     | 4.14   | 6.92    | 5.32      | 12.07| 19.24      | 11.94| 6.98   | 16.67     |
| EL      | East     | 6.02     | 5.03       | 8.11      | 9.18   | 8.84    | 6.91      | 19.04| 10.03      | 16.24| 6.85   | 28.06     |
| LP      | South    | 6.81     | 11.61      | 11.24     | 8.80   | 23.56   | 15.15     | 21.30| 16.70      | 14.78| 12.07   | 54.48     |
| LP      | North    | 4.19     | 7.76       | 9.42      | 7.71   | 20.47   | 15.45     | 21.30| 16.70      | 14.78| 12.07   | 54.48     |
| P       | South    | 16.45    | 23.02      | 12.00     | 17.96  | 28.25   | 20.27     | 14.13| 13.32      | 15.34| 12.32  | 21.35     |

1Late leaves are those that were produced in June and were located in the terminal third of a main shoot. Early leaves are those that were produced in April and were located in the basal third of a main shoot.
and south (micronutrients) sides of the tree (Table 6). For early-season leaves, the trends for variability were less clear. Petiole macronutrient concentrations tended to be more variable, but overall differences in variation were not great. Samples that consisted of both petiole and lamina typically had similar variation to samples with only lamina. The two cases in which the CV was different among tissue types was K (lower variation in lamina only samples) and Zn (lower variation in lamina + petiole samples). Although they identified differences in variability among tissue types in kiwifruit, the results of Sharma et al. (2005) do not match ours in all cases. Variation in sampling is likely also the result of genotype (species and cultivar) and environment and therefore would be expected to vary in different species and locations.

The data presented here are applicable to landscape as well as production settings. However, there are many more factors in a landscape (e.g., buildings and other plants) that could affect the microclimate of a given cardinal position within a tree. Although the leaf characteristics presented here may vary as a result of cultivar and location, the data suggest that sampling procedures for maple need not be too exacting. Although leaf age did greatly affect nutrient concentrations, most protocols suggest collecting recently matured foliage to determine any nutrient deficiencies that may be present and lead to reduced growth in the next season (Jones, 2001). Within the late-season growth, position did not greatly affect nutrient concentration, although samples should probably be collected from the south side of the tree, because the south side had the highest concentrations of N and Ca. Furthermore, the south and east sides of the tree tended to have lower variation within samples for macro- and micronutrients, respectively. It also appears that inclusion of the petiole in leaf nutrient analysis samples does not greatly affect the reported concentrations and therefore can be included. Most importantly, growers should strive for consistency in how they sample their plants for nutrient analysis, so seasonal trends can be used to make important fertilization decisions.

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