ELECTRICAL CAPACITANCE AS A PREDICTOR OF ROOT DRY WEIGHT IN SHRUB WILLOW (Salix; Salicaceae) PARENTS AND PROGENY

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• Premise of the study: Root biomass is an important trait often disregarded in woody perennial selection due to the challenge and expense of accurately and efficiently measuring large populations. In this study, we aim to develop a simple method that can predict root dry weight within a diverse shrub willow (Salix) breeding population representing species hybrids and their parents using root electrical capacitance (REC).

• Methods: The REC method was tested on plants started from cuttings and grown in pots with potting mix in the greenhouse for 11 wk to assess the relationship of REC with 24 biomass traits and its usefulness in allometric models for root and stem dry biomass.

• Results: Strong linear and positive correlations were found between REC and root dry biomass ($r = 0.88$). The total proportion of variance of root and stem dry biomass explained by predictors in multiple regression was 85% and 69%, respectively. The relative importance of predictor variables in allometric models was dominated by the contribution of REC.

• Discussion: This work provides an efficient and nondestructive technique to indirectly quantify root biomass of genetically diverse shrub willow progeny, which has great promise for selection of genotypes with varying root biomass and for the accurate estimation of belowground carbon sequestration.

Key words: bioenergy; biomass; breeding; phenotype; selection; short-rotation coppice.

The measurement of belowground traits of woody perennials has been the subject of renewed interest in recent years, because root biomass and architecture are critical for drought tolerance and for long-term carbon sequestration (Ericsson et al., 1996). Measurement of root biomass is a tremendously difficult and painstaking task in woody perennial breeding programs, as it requires careful washing, filtering, drying, and weighing fine root tissues, and thus is often avoided. For decades, electrical capacitance has been used to estimate the aboveground biomass of herbaceous perennials (Currie et al., 1987) and forest plantations (Lekas et al., 1990), but few root electrical capacitance (REC) studies have focused on clonal woody perennial plants as a model for predicting root biomass. Previous work on the estimation of root biomass using the REC method has primarily focused on hydroponically grown seedlings (Rajkai et al., 2005; Repo et al., 2005; Cao et al., 2011; Dietrich et al., 2012; Cseresnyés et al., 2013; Kormanek and Tomasz, 2015) or a limited number of genotypes (Whitlow et al., 1992; Pitre et al., 2010).

Dalton (1995) provided the first and most widely accepted conceptual model for using electrical capacitance as an in situ measurement for assessing root development. Electrical capacitance is measured in farads (F) and is considered the ability of a body (i.e., plant root tissue) to store an electrical charge, such that when charged with one coulomb (C) of energy, there is a potential difference of one volt (V), where $F = CV$. His method assumes that root capacitance is equivalent to a parallel resistance-capacitance circuit formed by the interface between soil and water and the root surface; or simply, that roots are equivalent to cylindrical capacitors where the epidermis and xylem are external and internal electrodes, respectively. Capacitance is formed by the polarization and relaxation of root membranes, which promotes fluctuations in amplitude and phase of the AC signal (Repo et al., 2000). This root–soil interface exhibits a capacitance proportional to the size of the membranes.

What exactly REC measures is debatable. Using hydroponically grown barley seedlings, Dietrich et al. (2012) argued that capacitance is linearly correlated with the sum of root cross-sectional areas at the solution surface and inversely related to the distance between the plant electrode and the solution surface. Likewise, the capacitance of barley is not determined by actual root biomass but by the cross-sectional area of roots at the solution surface (Dietrich et al., 2013). The distinction between this model and Dalton’s is that Dalton generalized roots to be cylindrical capacitors acting in parallel, whereas Dietrich argued that the capacitances of tissues along an unbranched root can be considered to be connected in series and the entire root system, in parallel. Although Repo et al. (2005) found a strong linear relationship between the cross-sectional area and the resistance of...
the stem, there was no difference in the relationship between resistance and cross-sectional area with or without the roots attached. Ellis et al. (2013) found little evidence that capacitance alone could be related to root mass, but these measurements could be used together with approximations of average root tissue fresh density to estimate coarse root length.

Dietrich et al. (2012) provides good evidence to modify the conceptual framework of Dalton’s original model, yet all analyses were based on fresh root mass of a hydroponically grown herbaceous annual. Compared to dry mass, wet mass can be extremely variable and heavily dependent on environmental factors (Lam et al., 2008) and is not an ideal estimate of root biomass for shrub willow. Furthermore, the compositional differences and developmental stage between seedling and mature, fully developed root systems cannot be ignored.

Here, we simply assume the Dalton model using a diverse breeding population of shrub willow (Salix; Salicaceae), with no assumptions concerning root morphology or architecture. Shrub willow has been bred as an energy crop in Europe since the early 1970s with the goal of producing fast-growing bioenergy feedstock cultivars that are high-yielding, genetically diverse, pest and disease resistant, and able to grow on marginal land without competing with food crops (Karp et al., 2011; Smart and Cameron, 2012). Shrub willow are dioecious riparian species commonly found near riverbanks and streambeds and are well adapted to the hypoxic conditions of wetlands (Kuzovkina et al., 2008). More than 350 species have been described in Salix L. (Argus, 2005; Lauron-Moreau et al., 2015) and have tremendous ecological amplitude, found within marginal and riparian habitats from the arctic plains to the subtropics (Kuzovkina et al., 2008). As the challenge to meet global energy objectives increases (Kriegl et al., 2014) along with global temperatures (Walther et al., 2002; Whiteman et al., 2013), the demand for low-input renewable bioproducts (Rose et al., 2014) gives shrub willow great potential as a competitive bioenergy feedstock for biomass production and conversion to biofuels to offset fossil fuel usage (Bonosi et al., 2013).

Our main objectives of this study were: (1) to investigate the relationship between electrical capacitance and root dry weight in diverse shrub willow families, (2) to contrast REC of parent genotypes and their hybrid progeny, (3) to study the relationship between REC and above- and belowground biomass traits, and (4) to evaluate the relative importance of REC in allometric models for the prediction of root and stem dry weight.

MATERIALS AND METHODS

Plant material—Parent genotypes and randomly chosen progeny of Salix purpurea L. (2n = 2x = 38), S. miyabeana Seemen (2n = 4x = 76), and S. viminalis L. (2n = 2x = 38) species crosses were grown from stem cuttings (20 cm) in 12-L plastic pots with moist-moss-based potting mix (Fafard, Agawam, Massachusetts, USA) to evaluate growth traits under greenhouse conditions over an 11-wk period. Families consisted of 12 progeny individuals and their parents from diploid interspecific S. purpurea × F1, and F2 crosses, two reciprocal diploid interspecific crosses (S. purpurea × S. viminalis), three triploid interspecific crosses (S. purpurea × S. miyabeana, S. miyabeana × S. viminalis, and S. viminalis × S. miyabeana), and an intraspecific tetraploid cross of S. miyabeana for a total of 104 genotypes (Table 1). One exception is that one of the parents of the S. miyabeana family (SX64, male parent of 425) was not included. Plot was defined as a single cut-in-the-stem, there was no difference in the relationship between resistance and cross-sectional area with or without the roots attached. Ellis et al. (2013) found little evidence that capacitance alone could be related to root mass, but these measurements could be used together with approximations of average root tissue fresh density to estimate coarse root length.

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Table 1. Description of family, pedigree, and generation in intraspecific and interspecific crosses of shrub willow (Salix; Salicaceae).

| Family ID | Female parent | Male parent | Pedigree | Ploidy |
|-----------|---------------|-------------|----------|--------|
| 082       | 94006         | 94001       | S. purpurea × S. purpurea | 2x     |
| 317       | ‘Wolcott’     | ‘Fish Creek’| S. purpurea × S. purpurea | 2x     |
| 407       | 94006         | ‘Jorr’      | S. purpurea × S. viminalis | 2x     |
| 421       | 07-MBG-5027   | S. purpurea × S. viminalis | 2x     |
| 415       | 94006         | 01-200-003  | S. purpurea × S. miyabeana | 3x     |
| 423       | 07-MBG-5027   | 01-200-003  | S. viminalis × S. miyabeana | 3x     |
| 430       | 01-200-006    | ‘Jorr’      | S. miyabeana × S. viminalis | 3x     |
| 425       | 01-200-006    | SX64        | S. miyabeana × S. miyabeana | 4x     |

Table 2. List of biomass-related variables including their descriptions and units.

| Trait abbreviation | Description          | Units | Measured (WAP) |
|--------------------|----------------------|-------|---------------|
| Aboveground        |                      |       |               |
| SDW                | Stem dry weight      | g plant⁻¹ | 11 |
| SSL                | Sum stem length      | cm     | 2–11 |
| MSL                | Mean stem length     | cm     | 2–11 |
| MXL                | Maximum stem length  | cm     | 2–11 |
| MSA                | Mean stem area       | cm²    | 11 |
| SSA                | Sum stem area        | cm     | 11 |
| RGR                | Relative growth rate | cm d⁻¹ | 3–11 |
| PSN                | Primary stem number  | #      | 11 |
| ASN                | Axial stem number    | #      | 5, 10 |
| LDW                | Leaf dry weight      | g plant⁻¹ | 11 |
| SPD                | SPAD                 | SPAD units | 2, 4, 10, 11 |
| PHE                | Vegetative phenology | 0–5   | 2–3 |
| MSD                | Mean stem diameter   | cm     | 11 |
| SSD                | Sum stem diameter    | cm     | 11 |
| LFA                | Leaf area            | cm²    | 11 |
| SLA                | Specific leaf area   | cm² g⁻¹ | 10 |
| LMA                | Leaf mass per area   | g cm⁻² | 10 |
| Belowground        |                      |       |               |
| REC                | Root electrical capacitance | nF | 11 |
| RDW                | Root dry weight      | g plant⁻¹ | 11 |
| CDIA               | Cutting diameter     | cm     | 11 |
| CDW                | Cutting dry weight   | g plant⁻¹ | 11 |
| RDI                | Mean root diameter   | cm     | 11 |
| PRN                | Primary root number  | #      | 11 |
| SRN                | Secondary root number | # | 11 |
| TRN                | Total root number    | #      | 11 |

Note: WAP = weeks after planting.

RESULTS

Family variation in REC and biomass production—The means of REC significantly differed by up to 34% among the eight full-sib families (Fig. 1, Appendix S1). The triploid family 430 (S. miyabeana × S. viminalis) displayed the lowest REC mean (182.8 nF), while the triploid family 415 (S. purpurea × S. miyabeana) has the lowest REC with a mean of 120.8 nF (Fig. 1, Appendix S1). The effect of clone within family accounted for a majority of the variance of REC. In addition, the effect of the female parent was almost twice that of the male parent.
Combinations of parents with high GCA values should theoretically produce progeny with high absolute trait values. In absolute terms, the *S. purpurea* female clone 94006 had the greatest GCA of all parents. Of the families analyzed, MPH for REC was observed in the families 421, 423, and 430 but not in families 082 (F1 *S. purpurea × S. purpurea*), 317 (F2 *S. purpurea × S. purpurea*), 415 (F2 *S. purpurea × S. miyabeana*), and 407 (F2 *S. purpurea × S. viminalis*) (Fig. 1). Because one of the parents of family 425 was not included, heterosis was not calculated for that family. The female and male *S. viminalis* (07-MBG-5027 × ‘Jorr’) and the female *S. miyabeana* (01-200-006) parent clones had the greatest GCA of family parents (Appendix S2) contributing to progeny REC. Midparent heterosis for REC was the most dramatic for interspecific crosses of these parents, i.e., families 430 (*t* = 4.09, *P* < 0.001) (01-200-006 × ‘Jorr’), 423 (*t* = 3.56, *P* = 0.003) (07-MBG-5027 × 01-200-003), and 421 (*t* = 2.59, *P* = 0.02) (07-MBG-5027 × 94,001) (Appendix S1).

**Model selection and predictor comparison**—For all traits analyzed, significant REC correlation coefficients (*P* < 0.05) ranged from 0.11 (SPAD) to 0.88 (RDW). The REC trait was positively correlated with many above- and belowground biomass traits (Table 2, Fig. 2). Aboveground traits that most strongly correlate with REC were SDW, LDW, SSL, and MSA (Table 3). Belowground traits that most strongly correlated with REC were RDW, CDW, and CDIA (Table 3). Owing to the significant contribution of REC and SRN predictor variables, the reduced model, RDW ~ REC + CDIA + PRN + SRN, explains a substantial portion of the variation in RDW (multiple *R*² = 0.85, *P* < 0.001) (Appendix S3). Relative importance metrics for SDW predictor variables did not vary greatly among model orderings (i.e., rank); in contrast to within-trait *R*² and bootstrapped CIs (boot = 1000, Bonferroni CI = 95%), the magnitude of the difference in variation explained in the predictors did vary significantly (Fig. 3). The proportion of variance that could be explained using the seven predictors in the final allometric model, SDW ~ REC + SSL + MSL + SSD + ASN + PSN + SPD, was 68.5% (Appendix S4). A large proportion of the variance is explained by the relative individual contributions of REC (*R*² = 0.42), SSL (*R*² = 0.14), and MSL (*R*² = 0.15). When REC is not included as a predictor variable in multiple regression, only 55.9% of the variation in SDW could be explained by the remaining variables—a difference of 12.6%. In each model index, REC was found to be significantly different compared to all other predictor variables and explained the greatest proportion of variance in each model index.

**DISCUSSION**

There are a number of key traits that could be selected in shrub willow breeding programs if a rapid method for phenotyping root traits was available, including rate of rooting establishment, ability to compete with weeds, drought tolerance, and total carbon sequestration ability (Pacaldeo et al., 2013). The partitioning of photoassimilate from foliage to stem and root biomass is largely dependent upon a complex network of photosynthetic and transport processes (Dickson, 1989; Ericsson et al., 1996), as well as other morphological and environmental factors (Samuelson and Kelly, 1996). Rather than assume that the root:shoot ratio is constant among segregating progeny, it is likely there are genotypic differences in the accumulation of root biomass in proportion to aboveground biomass. While it is true that the quantitative nature of REC does not offer direct insight into root morphology or architecture, the specific morphologically based variances in REC need not be known if it is still a good predictor of RDW.
Increased vigor can be observed in intraspecific crosses of shrub willow, but the effect is generally more dramatic in the progeny of interspecific crosses (Serapiglia et al., 2014), especially in triploid progeny derived from the hybridization of tetraploid and diploid parents (Serapiglia et al., 2015; Fabio et al., 2016). Likewise, we show that the interspecific families 421, 423, and

### Table 3. Pearson correlation coefficients, their confidence intervals, and significance of above- and belowground traits with root electrical capacitance (REC) and root dry weight (RDW). See Table 2 for trait definitions.

| Trait | REC | CI       | P value | RDW | CI       | P value |
|-------|-----|----------|---------|-----|----------|---------|
| Aboveground |     |          |         |     |          |         |
| SDW   | 0.72| 0.66–0.75| <0.001  | 0.52| 0.29–0.75| <0.001  |
| LDW   | 0.56| 0.49–0.62| <0.001  | 0.78| 0.51–0.91| <0.001  |
| SSL   | 0.53| 0.45–0.59| <0.001  | 0.59| 0.22–0.82| 0.005   |
| MSL   | 0.37| 0.28–0.45| <0.001  | 0.69| 0.35–0.86| <0.001  |
| MXL   | 0.44| 0.35–0.51| <0.001  | 0.78| 0.52–0.91| <0.001  |
| RGR   | 0.42| 0.33–0.49| <0.001  | 0.33| 0.27–0.44| 0.005   |
| MSA   | 0.23| 0.14–0.32| <0.001  | 0.31| —        | 0.178   |
| SSA   | 0.39| 0.31–0.46| <0.001  | 0.20| —        | 0.406   |
| LFA   | 0.09| —        | 0.092   | 0.32| —        | 0.159   |
| PSN   | 0.22| 0.13–0.31| <0.001  | 0.23| —        | 0.310   |
| ASN   | 0.19| 0.10–0.28| <0.001  | 0.64| 0.29–0.85| 0.002   |
| SPD   | −0.11| 0.01–0.20| <0.001  | −0.34| —        | 0.139   |
| PHE   | 0.38| 0.29–0.46| <0.001  | 0.58| 0.18–0.81| 0.007   |
| MSD   | 0.13| 0.03–0.22| <0.001  | 0.33| —        | 0.148   |
| SSD   | 0.39| 0.31–0.47| 0.0091  | 0.19| —        | 0.414   |
| SLA   | −0.23| 0.13–0.31| <0.001  | −0.56| 0.15–0.80| 0.010   |
| LMA   | 0.24| 0.20–0.30| <0.001  | 0.59| 0.13–0.79| 0.007   |
| Belowground |     |          |         |     |          |         |
| RDW   | 0.88| 0.71–0.95| <0.001  | 0.88| 0.71–0.95| <0.001  |
| CDIA  | 0.65| 0.30–0.85| 0.001   | 0.67| 0.33–0.86| 0.001   |
| CDW   | 0.67| 0.33–0.86| <0.001  | 0.70| 0.37–0.84| <0.001  |
| RDIA  | 0.20| 0.27–0.59| 0.002   | 0.05| —        | 0.982   |
| PRN   | 0.64| 0.29–0.84| 0.016   | 0.50| 0.08–0.77| 0.023   |
| TRN   | 0.59| 0.20–0.81| 0.041   | 0.53| 0.12–0.79| 0.005   |
| SRN   | 0.34| 0.12–0.67| 0.006   | 0.45| 0.03–0.74| 0.041   |
thereby providing a competitive advantage for species with high LMA in nutrient-poor soils (Poorter et al., 2009). In this study, LFA was weakly correlated with RDW (\(R^2 = 0.32, P = 0.16\)) and only significantly correlated with REC (\(R^2 = 0.40, P = 0.005\)) in the diploid family 407 (\(S. purpurea\) × \(S. viminalis\)). The weak relationship between LFA and RDW can potentially be explained by high genotypic variances in carbon partitioning in leaves (Weraduwage et al., 2015) or a relatively small sample size.

Both REC and RDW strongly correlate to SDW. Early prediction of harvestable biomass yield is extremely important for the selection of high-performance woody perennial crops like shrub willow. If RDW contributes a substantial portion of SDW variance, it is exemplified by the REC technique. In all multiple regression model orderings, REC has shown to be the best predictor of SDW among all traits attributing to each model. Although LDW was strongly correlated with SDW (\(R^2 = 0.81, P < 0.001\)), it was excluded as a predictor variable in multiple regression analyses because it is a destructive measurement and not manageable to collect leaf biomass in a field setting. As far as we know, this is the first attempt using REC as a predictor variable in allometric models for yield.

Estimation of root biomass relative growth rates may be modeled from multitemporal data with minimal effort. The root system of shrub willow typically resides in the upper 20–30 cm of the soil and fine roots in the upper 10 cm (Pacaldo et al., 2013), which may find worthy implementation of REC as a long-term indicator of root development in research field trials. It may be that the proximal morphology of adventitious roots from the
cutting surface may provide the best explanation for the strong and positive correlation of REC with RDW. For the reason that shrub willow does not produce a large tap root from a cutting, it is possible that the proximity of the capacitance meter lead in the soil and the proximal root system provides a strong signal to the extent that a majority of the variance of root biomass can be explained by REC. However, comparisons of both seedlings and cuttings of shrub willow families must be conducted to determine if there are significant differences associated with root morphology impacting the prediction of RDW with REC. The effect of aboveground biomass on REC should be considered while conducting REC on a large-scale field study, and curves must be recalibrated accordingly. For the reason that aboveground biomass was harvested prior to taking REC measurements in this study, any confounding factors that may have been introduced into analyses were avoided. For instance, the difficulty of maintaining equivalent moisture content across genotypes is relative to the transpiration rate of respective genotypes, which were extremely diverse. Because the moisture content needed to be stable across plots, as noted in Dalton (1995), REC was measured after aboveground biomass was harvested, ensuring minimal water loss from pots over time. In the application of REC to the measurement of a large-scale field experiment, one would also need to measure moisture content adjacent to each REC measurement to use as a covariate in order to account for error associated with local variation in soil moisture content.

In addition to estimating RDW, there is also potential to investigate the relationship of REC with the occurrence and distribution of ectomycorrhizal and arbuscular mycorrhizal populations among short-rotation plantations and natural stands of shrub willow (Dhillion, 1994; Cseresnys et al., 2013). Repo et al. (2016) implemented a PC-based method using electrical impedance spectra to assess the hardness of mycorrhizal and nonmycorrhizal roots of Scots pine seedlings. Although there were no significant differences due to mycorrhizal treatments, Repo et al. (2016) found differences between treatment temperatures. Whether the accumulation of mycorrhizas is genotype-specific or impacts REC (Cseresnys et al., 2016) in Salix spp. has not yet been characterized. It would be worthwhile to measure REC and sample mycorrhizae concurrently in a field setting to analyze the effects of altered environmental conditions on root growth and biomass yield.

The REC method provides an efficient, reproducible, and nondestructive alternative to more traditional “shovel-omics” techniques. However, the efficiency of REC must still be improved and the equipment itself scaled up to withstand heterogeneous soil profiles and thick stems on stolons with multiple stems of mature plants in the field. For instance, Ellis et al. (2013) used the electrical capacitance 4T method (resistor and capacitor in series) as an indicator of root length in Pinus L., Eucalyptus L’Hér., and Corymbia K. D. Hill & L. A. S. Johnson forest tree plantations, resolving capacitance somewhat related to root mass within species but a poor indicator of diameter at breast height, root mass, or root length across species, age, or sites. In this study, we selected a relatively small sample size for validation of REC and RDW because of the rigor and time expense associated with carefully washing and retrieving fine and coarse roots from mature root masses in a short period of time. Only a few days after harvesting stem and leaf biomass from pots, new shoots began to emerge from their stools. Moreover, washing root samples over a one- or two-week period could introduce unwanted error into statistical analyses, thereby impacting the relationship between REC collected at harvest and RDW, as well as correlations with morphological and physiological traits collected at the termination of the study. Technological improvements in field-based root phenotyping (Rautenbach et al., 2013; Meister et al., 2014) will hopefully allow for high-throughput quantification of root architecture and biomass at a reasonable efficiency and cost.

Compared to annual crops, it is likely that the electrical capacitances of perennial shrub willows will change over time as a result of root suberization, repeated coppicing, and seasonal variation of biotic and abiotic stressors. As implied, future work must be accomplished to determine if the strong relationship between REC and biomass-related traits on second- and third-year growth are equally as strong as in the first year. It is likely that over multiple coppice cycles, environment will play an important role in determining the genotypic stability of this trait in shrub willow. Nevertheless, the strength of REC prediction estimates of above- and belowground dry biomass is sufficient to conclude that it is not only valuable but is adaptable across *Salix* species, ploidy level, and hybrid pedigree.

**LITERATURE CITED**

**ALFONS, A. 2012.** cvTools: Cross-validation tools for regression models. Comprehensive R Archive Network. Website https://CRAN.R-project.org/package=cvTools [accessed 11 July 2016].

**ARGUS, G. W. 2005.** Guide to the identification of the genus *Salix* (willow) in New England and New York. Delta Institute of Natural History, Bowdoin, Maine, USA.

**BATES, D., M. MACHLER, B. BOLKER, AND S. WALKER. 2015.** lm4: Mixed-effects modeling with R. Fitting linear mixed-effects models using lm4. *Journal of Statistical Software* 67: 1–48.

**BONSEL, L., L. GHILARDINI, AND M. WEHL. 2013.** Towards making willows potential bio-resources in the South: Northern *Salix* hybrids can cope with warm and dry climate when irrigated. *Biomass and Bioenergy* 51: 136–144.

**CAO, Y., T. REPO, R. SILVENNOINEN, T. LEHTO, AND P. PELKONEN. 2011.** Analysis of the willow root system by electrical impedance spectroscopy. *Journal of Experimental Botany* 62: 351–358.

**CSERESNYÉS, I., T. TÁKACS, K. R. VEGH, A. ANTON, AND K. RAJKAI. 2013.** Electrical impedance and capacitance method: A new approach for detection of functional aspects of arbucular mycorrhizal colonization in maize. *European Journal of Soil Biology* 54: 25–31.

**CSERESNYÉS, I., K. RAJKAI, AND T. TÁKACS. 2016.** Indirect monitoring of root activity in soybean cultivars under contrasting moisture regimes by measuring electrical capacitance. *Acta Physiologiae Plantarum* 38: 121.

**CUNNEFF, J., S. J. PURDY, T. J. P. BARRACLOUGH, M. CASTLE, A. L. MADISON, L. E. JONES, I. F. SHIELD, ET AL. 2015.** High yielding biomass genotypes of willow (Salix spp.) show differences in below ground biomass allocation. *Biomass and Bioenergy* 80: 114–127.

**CURIEIL, P. O., T. O. HILKEN, AND R. S. WHITE. 1987.** Evaluation of a single probe capacitance meter for estimating herbage yield. *Journal of Range Management* 40: 537–541.

**DALTON, F. N. 1995.** In-situ root extent measurements by electrical capacitance methods. *Plant and Soil* 173: 157–165.

**DILLION, S. S. 1994.** Ectomycorrhizae, arbuscular mycorrhizae and *Rhizoctonia* sp. of alpine and boreal Salix spp. in Norway. *Arctic and Alpine Research* 26: 304–307.

**DICKSON, R. E. 1989.** Carbon and nitrogen allocation in trees. *Annals of Forest Science* 46: 631s–647s.

**DIETRICH, R. C., A. G. BENDOUGH, H. G. JONES, AND P. J. WHITE. 2012.** A new physical interpretation of plant root capacitance. *Journal of Experimental Botany* 63: 6149–6159.

**DIETRICH, R. C., A. G. BENDOUGH, H. G. JONES, AND P. J. WHITE. 2013.** Can root electrical capacitance be used to predict root mass in soil? *Annals of Botany* 112: 457–464.
Carlson and Smart—Electrical capacitance of roots

PRESTON, G. M., R. A. McBride, J. BRYAN, and M. CANDIDO. 2004. Estimating root mass in young hybrid poplar trees using the electrical capacitance method. Agroforestry Systems 60: 305–309.

R DEVELOPMENT CORE TEAM. 2011. R: A language and environment for statistical computing. R foundation for statistical computing, 1–409. Vienna, Austria. Website https://www.R-project.org/ [accessed 11 July 2016].

RAKAI, K., K. R. V. ÉGH, and T. NACSA. 2005. Electrical capacitance of roots in relation to plant electrodes, measuring frequency and root media. Acta Agronómica Hungarica 53: 197–210.

RauTENbACh, C., R. F. MÜDDLE, X. YANG, M. C. MELAaEN, and B. M. HALVORESEN. 2013. A comparative study between electrical capacitance tomography and time-resolved X-ray tomography. Flow Measurement and Instrumentation 30: 34–44.

RePo, T., G. ZHANG, A. RYVPPO, and R. RIKAL. 2000. The electrical impedance spectroscopy of Scots pine (Pinus sylvestris L.) shoots in relation to cold acclimation. Journal of Experimental Botany 51: 2095–2107.

RePo, T., J. LAUKKANEN, and R. SILVENNOINEN. 2005. Measurement of the tree root growth using electrical impedance spectroscopy. Silva Fennica 39: 159–166.

RePo, T., A. KORHONEN, T. LEHTO, and R. SILVENNOINEN. 2016. Assessment of frost damage in mycorrhizal and non-mycorrhizal roots of Scots pine seedlings using classification analysis of their electrical impedance spectra. Trees (Berlin) 30: 483–495.

ROse, S. K., E. Kriegler, R. BRIAS, K. CALVIN, A. POPP, D. P. VAN VuUREN, and J. WEYANT. 2014. Bioenergy in energy transformation and climate management. Climatic Change 123: 477–493.

RySER, P. 1996. The importance of tissue density for growth and life span of leaves and roots: A comparison of five ecologically contrasting grasses. Functional Ecology 10: 717–723.

SAMUELSON, L. J., and J. M. KELLY. 1996. Carbon partitioning and allocation in northern red oak seedlings and mature trees in response to ozone. Tree Physiology 16: 853–858.

SERAPIGLIA, M. J., F. E. GOUKER, and L. B. SMART. 2014. Early selection of novel triploid hybrids of shrub willow with improved biomass yield relative to diploids. BMC Plant Biology 14: 74.

SERAPIGLIA, M. J., F. E. GOUKER, J. F. HART, F. UNDA, S. D. MANSFIELD, A. J. STIPANOVIĆ, and L. B. SMART. 2015. Ploidy level affects important biomass traits of novel shrub willow (Salix) hybrids. BioEnergy Research 8: 259–269.

SMART, L. B., and K. D. CAMERON. 2012. Shrub willow. In C. KOLE, S. JOSHI S., and D. SHONNAARD [eds.], Handbook of bioenergy crop plants, 687–708. Taylor and Francis Group, Boca Raton, Florida, USA.

WALther, G. R., E. POST, P. CONVEY, A. MENZEL, C. PARMESEAN, T. J. C. BEEBEE, J. M. PROMENTIN, et al. 2002. Ecological responses to recent climate change. Nature 416: 389–395.

WEzdllwAgA, S. M., J. CHEN, F. C. ANZIEU, A. MORALES, S. E. WEINE, and T. D. SHARKEY. 2015. The relationship between leaf area growth and biomass accumulation in Arabidopsis thaliana. Frontiers in Plant Science 6: 167.

WHITMAN, G., C. HOPE, and P. WADHAMS. 2013. Vast costs of Arctic change. Nature 499: 401–403.

WHITLOW, T. H., N. L. BASSEK, T. G. Rannay, and D. L. Reichert. 1992. An improved method for using electrolyte leakage to assess membrane competence in plant tissues. Plant Physiology 98: 198–205.