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WinRHIZO Technology for Measuring Morphological Traits of Bermudagrass Stolons

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ABSTRACT

WinRHIZO, a root-measuring system, may provide an alternative, reliable, and fast method to analyze stolon morphology of grass species. This study evaluated the possibility to use WinRHIZO technology to measure total length and average diameter of bermudagrass [*Cynodon dactylon* (L.) Pers.] stolons. The length and diameter of 70 stolons collected from four turf-type cultivars and a wild bermudagrass were measured with a ruler (length) and caliper (diameter), and using WinRHIZO technology, a scanner-based image analysis system. The scanned length closely predicted the manually measured one and can be successfully used to determine stolon length in samples with a significant amount of biomass. WinRHIZO technology overestimated diameter values when the whole stolon was scanned, while the diameter prediction was more precise when nodes were removed and only internodes were measured.

Core Ideas

- WinRHIZO technology may provide a method to analyze stolon morphology of grasses.
- The scanned length closely predicted the manually measured one.
- WinRHIZO technology overestimated diameter when the whole stolon was scanned.
- WinRHIZO technology prediction was precise when only internodes were measured.

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Stolons and rhizomes are elongated stems produced by many turfgrass species and feature nodes from which adventitious roots and shoots can form. These structures serve a wide range of functions, including being a key plant propagation strategy (i.e., sprigging) in some species and can also significantly impact recuperative ability of turfgrasses damaged by biotic or abiotic stress. These morphological structures are also major carbohydrates storage organs, in that the carbohydrates accumulated in stolons and rhizomes are essential for the plant to survive under stress periods and to recover after stress cessation (Dunn and Nelson, 1974; Di Paola and Beard, 1992; Fry et al., 1993; Gatschet et al., 1994; Schiavon et al., 2016).

The most widespread turfgrass species that features both stolons and rhizomes is bermudagrass, described as an aggressive warm-season turfgrass species (Harivandi, 1986; Avcıoğlu, 1997). It has been reported that stolon morphological traits of bermudagrass are genetically controlled (de Kroon et al., 1994), and their influence on turfgrass quality and cold tolerance have been widely studied (Dudeck and Murdoch, 1998; Roche and Loch, 2005; Hensler et al., 1998; Anderson et al., 2007). High stolon density is of crucial importance for cold hardiness and spring recovery from winter injury in many warm-season species (Hensler et al., 1998; Trenholm et al., 2000; Anderson et al., 2007; Rimi et al., 2013b). Cultivar selection of bermudagrass, especially in the transition zone, takes into account stolon density since it has been well correlated to cold hardiness (Rimi et al., 2013b; Dunn and Diesberg, 2004). Moreover, stolon density is involved in other aspects considered important in bermudagrass cultivar selection such as turfgrass quality, establishment vigor, and divot recovery (Patton et al., 2008).

Study of stolon and rhizome traits in a mature turfgrass is often based on the measurement of internode length and diameter, and mass dry weight (Lulli et al., 2012; Munshaw et al., 2001; Rimi et al., 2013a, 2013b). Several studies underlined the greater interest of stolon dry weight in optimum establishment seeding rates (Munshaw et al., 2001) and cold tolerance (Rimi et al., 2013b; Schiavon et al., 2016) of warm season grasses, while little attention is given to total stolon length and diameter. Rimi et al. (2013b) and Schiavon et al. (2016) observed significant differences in stolon dry weight and carbohydrate content of bermudagrass cultivars and suggested that high stolon dry weight and high carbohydrates influence cold tolerance and accelerate spring green-up. While stolon dry weight can be easily measured by processing the stolons in a drying oven, internode length and diameter are conventionally measured using a ruler or caliper and require

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significant labor and time costs. Thus, diameter and length of internodes are usually used for morphological characterization of spaced plants and are measured on a limited number of stolons (i.e., Lulli et al., 2012; Taliaferro et al., 2006).

Although length and diameter are not the most widely used parameters in the analysis of stolon morphology, they are commonly used in the characterization of root systems. Root length density (root length per soil volume) and average diameter are commonly estimated in turfgrass studies as root morphological descriptors through the use of WinRHIZO technology (Regent Instrument Inc., Ville de Québec, QC Canada) (i.e., Barnes et al., 2014; Macolino et al., 2012; Rimi et al., 2012). As the length of stolons per unit of surface and the average dimeter are essential parameters for the morphological characterization of stems in stoloniferous species, the use of WinRHIZO technology may provide a method to analyze these traits more completely and faster than the current method. Furthermore, WinRHIZO technology may also be more convenient because it overcomes measurement errors due to human mistakes and can be used for large sample sizes, thus increasing statistical precision. WinRHIZO is an image analysis system specifically designed for measurement of washed roots in different forms. The scanner used for image acquisition has a specific lighting and background system to avoiding artifacts produced by inconsistent light. The system is extremely easy to use, and does not need to be recalibrated each time the optical setup or the resolution is changed (Arsenault et al., 1995).

WinRHIZO, which is typically used for root measurements, may also provide an alternative, reliable, and fast method to analyze stolon morphology of grass species. The overall objective of this study was to evaluate if WinRHIZO technology could be used to measure total length and average diameter of bermudagrass stolons. Stolon length and diameter data were obtained from traditional measurements and were correlated with the respective data estimated using the WinRHIZO software.

MATERIALS AND METHODS

The experiment was conducted at the Experimental Agricultural Farm of Padova University in Legnaro, northeastern Italy (45°20′ N, 11°57′ E, and elevation 8 m). The location has a humid subtropical climate, with an annual rainfall of 820 mm mostly distributed from April to November and a mean annual temperature of 12.3°C (based on a 40-yr series). The soil at the site was a coarse-silty, mixed, mesic, Oxyaquic Eutrudept (Morari, 2006) containing 17.3% clay, 64.9% silt, and 17.9% sand, with a pH of 8.1, 2.57% organic matter, a C/N ratio of 11.96, an Olsen P content of 5.1 mg kg^{-1}, and an exchangeable K content of 165.9 mg kg^{-1} (buffered $BaCl_2$ method).

Seventy stolons were randomly collected in existing turfgrass plots arranged in a randomized complete block design with three replicates and from wild bermudagrass plants growing near the plots. The turfgrass plots were established in May 2013 in Legnaro using two seeded cultivars (LaPaloma and Yukon) and two vegetative cultivars (Patriot and Tifway) of bermudagrass. Plots were maintained without irrigation, and fertilization was applied monthly from May to August at a rate of 5 g m^{-2} of N. Mowing was performed weekly during the growing season using a rotary mower (HRD536; Honda Europe Power Equipment, Ormes, France) and clippings were removed. The 70 stolons (14 stolons for each cultivar randomly collected in plots, plus 14 stolons of wild bermudagrass) were collected in September 2013. Successively, all the stolons were hand cleaned by removing the leaves, shoots, and roots before any measurement. Diameter and length of all internodes were measured with a caliper and ruler, respectively, and the number of nodes for each stolon was counted. The time necessary to clean and measure with the ruler and caliper three samples of 14 stolons for the cultivars, Patriot and Yukon, and the wild bermudagrass (42 total stolon) was recorded. Measured stolon diameter was calculated for each stolon as the mean of all internode diameters, and total stolon length was calculated as the sum of all internode lengths. Furthermore, total scanned length and scanned diameter of each stolon were measured using WinRHIZO version 2003b (Regent Instruments Inc., Quebec), while recording the time necessary to measure each stolon sample. Each stolon was then cut with scissors to separate internodes from nodes, and the internodes were used to estimate the scanned internodes diameter using WinRHIZO technology.

Statistical significances of correlations were evaluated and Pearson's correlation coefficients were calculated ($N = 70$) to determine the degree of association between measured and scanned length, measured and scanned stolon diameter, number of nodes and the absolute value of the difference between measured and scanned stolon diameter, and measured stolon diameter and scanned internode diameter. Analysis of variance was performed using SAS version 9.4 (SAS Institute, Cary, NC) on measured stolon diameter, scanned stolon diameter, and scanned internode diameter, to investigate the ability of the different measurements to separate cultivar differences. Data were transformed when necessary before ANOVA to achieve normality. Fisher's protected least significant difference test ($P = 0.05$) was used to identify significant differences among means.

RESULTS AND DISCUSSION

The stolon lengths measured with a ruler were used to calibrate the lengths estimated through WinRHIZO technology. The scanned length closely predicted the measured one (Fig. 1) as regression analysis indicated a high correlation between the two, with a slope of 1.03 and an intercept of 4.22. The stolon lengths measured with the ruler were underestimated by only 0.007%. Therefore, using the WinRHIZO technology for numerous stolons derived from a turfgrass sample, it is possible to easily and accurately quantify the total stolon length. Stolon abundance in a turfgrass sward is usually described by stolon mass density (mg cm^{-2}), which is measured after oven-drying (Munshaw

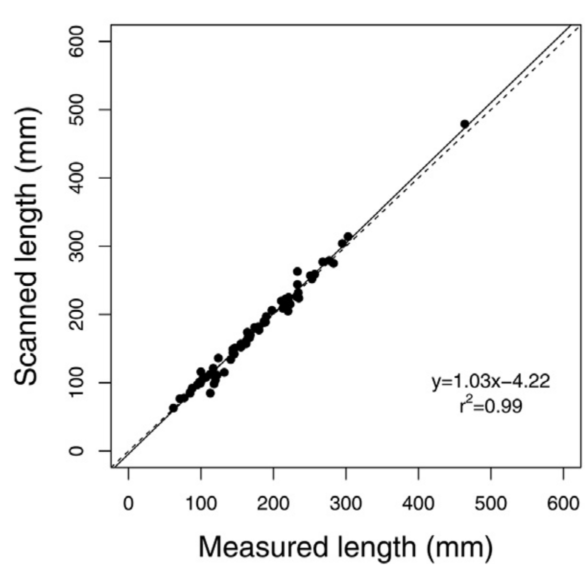

Fig. 1. Regression analysis of stolon length values measured with the ruler against values estimated with the WinRHIZO technology. Dashed line represents the 1:1 ratio.

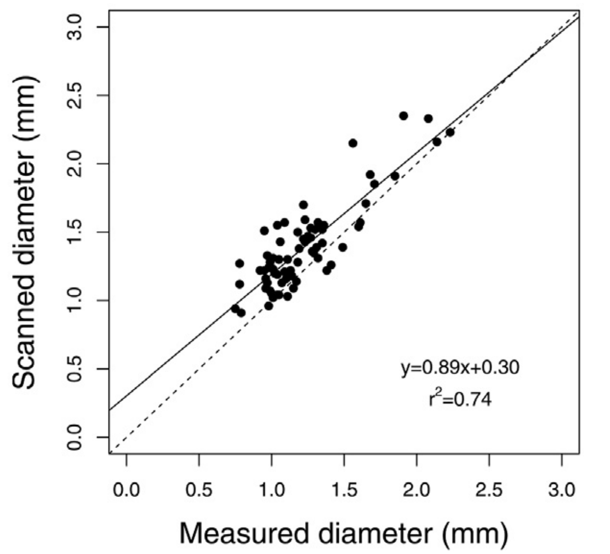

Fig. 2. Regression analysis of stolon diameter values measured with the caliper against values estimated with the WinRHIZO technology for the whole stolons. Dashed line represents the 1:1 ratio.

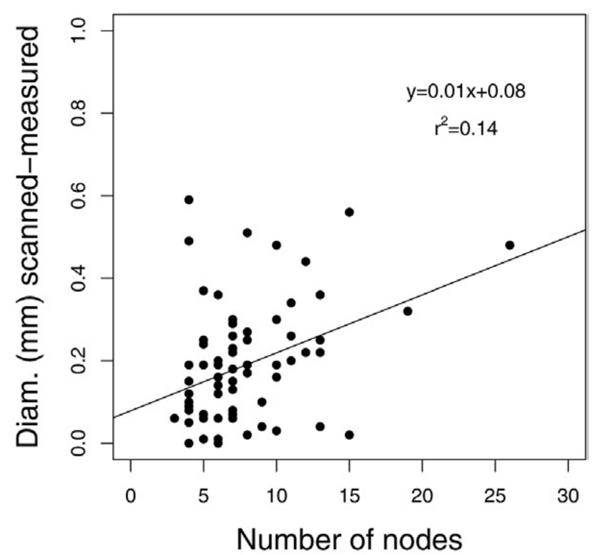

Fig. 3. Regression analysis of number of stolon nodes against absolute values of difference between stolon diameter estimated with the WinRHIZO technology and measured with the caliper.

et al., 2001; Rimi et al., 2013a; Schiavon et al., 2016). Similarly, stolon length can be used to calculate stolon length density (cm cm^{-2}), and both can be applied in analysis of samples with a large amount of biomass. Therefore, WinRHIZO technology can be successfully used to calculate stolon length density of a mature turfgrass in an efficient manner. The average time to clean 14 stolons by hand was 21 min 24 s and measuring their length and diameter with ruler and caliper took an average of 14 min 06 s. The scan and analysis of the stolon sample using WinRHIZO took an average of 11 min 00 s. As such, the time spent for complete analysis was similar for both methods, although WinRHIZO was slightly more time efficient than physical measurements. However, the WinRHIZO method allows a researcher to measure a large number of stolons in a single image analysis and the time difference between methods would likely become more apparent as sample size is increased.

Measured and scanned stolon diameter were also significantly correlated. The relationship between measured and scanned diameter was close to 1:1, indicating a good fit of the data (Fig. 2). However, the intercept indicated that WinRHIZO technology overestimated measured diameter values by 10%, especially for lower values. These results could be partially explained by the presence of stolon nodes that are scanned and used for calculating stolon diameter by the WinRHIZO software (ratio between total projection surface and total length), while they are not included in the caliper measurements. In samples with a large stolon mass, the number of nodes could impact stolon diameter estimations. The correlation between number of nodes and the difference between diameter values obtained by the two methods (measured and scanned) was significant but the low R^2 indicates that only 14% of the variation of this difference can be explained by the variations in the number of nodes (Fig. 3). The high significant correlation found between scanned internode diameter and measured stolon diameter, having a slope of 1.01 and an intercept nearly zero (Fig. 4), demonstrates that internode diameter can be accurately estimated through WinRHIZO technology as long as nodes are removed.

To evaluate the differences between the three methods of evaluating stolon diameter, we performed an analysis of variance on each method, analyzing the effect of cultivar on stolon diameter. The effect of cultivar was significant for all three methods, and the mean separation

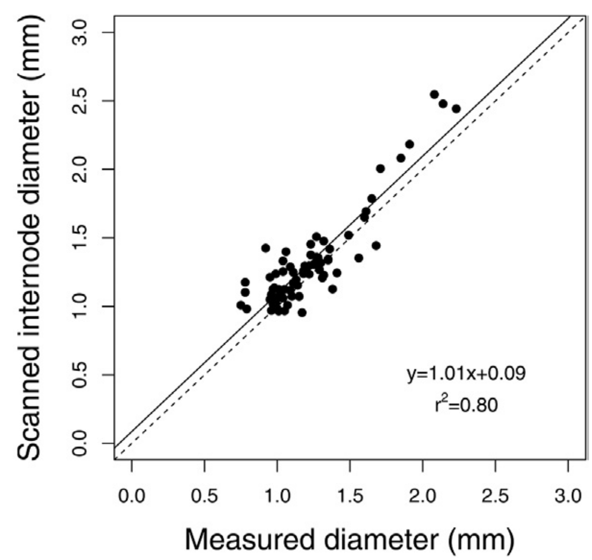

Fig. 4. Regression analysis of stolon diameter values measured with the caliper against values estimated with the WinRHIZO technology for internodes. Dashed line represents the 1:1 ratio.

Table 1. Diameter measured with caliper (measured stolon diameter), estimated with WinRHIZO technology for the whole stolon (scanned stolon diameter) and scanned with WinRHIZO technology for interdode (scanned internode diameter) of analyzed cultivars. Values with the same letter are not significantly different (Fisher's protected LSD test at the 0.05 probability level).

Bermudagrass cultivars	Measured stolon diameter	Scanned stolon diameter	Scanned internode diameter
		mm	
Wild	1.62a	1.87a	1.78a
Patriot	1.23b	1.40b	1.26b
Yukon	1.15bc	1.34b	1.26b
La Paloma	1.10bc	1.23bc	1.21bc
Tifway	1.05c	1.17c	1.08c

test gave similar results (Table 1). Means are separated in three groups where wild bermudagrass displayed the highest values and cultivar Tifway the lowest. This demonstrated that scanned stolon diameter values are comparable to measured stolon diameter and scanned internode diameter. However, since diameter of internodes is a widely used parameter for botanical description (Taliaferro et al., 2006; Rimi, 2012), we suggest that the scanned and measured diameter are descriptors of two different aspects: the first corresponds to mean diameter of the whole plant structure, including nodes, while the measured diameter only refers to the mean diameter of the internodes. Furthermore, the measurement of internode diameter using the WinRHIZO method should reduce the experimental error due to data entry than caliper measurements when applied on a large number of internodes, taking the same time of caliper measurement.

These results demonstrate that WinRHIZO technology can be effectively used to determine stolon length and diameter in spaced experiments and mature turfgrass stands. The use of WinRHIZO could permit larger samples, in both size or numbers, to be measured, providing a more robust estimation of stolon morphology than what can be measured with a ruler or caliper. It is also likely that similar approaches could be applied to the measurement of rhizomes in spreading grasses.

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