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 carbohydrate 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 turfgrass 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
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; Talafarro 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 diameter 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 avoid 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 technology.

**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...
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. 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.](image)

![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.](image)

![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.](image)

<table>
<thead>
<tr>
<th>Bermudagrass cultivars</th>
<th>Measured stolon diameter</th>
<th>Scanned stolon diameter</th>
<th>Scanned internode diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>1.62a</td>
<td>1.87a</td>
<td>1.78a</td>
</tr>
<tr>
<td>Patriot</td>
<td>1.23b</td>
<td>1.40b</td>
<td>1.26b</td>
</tr>
<tr>
<td>Yukon</td>
<td>1.15bc</td>
<td>1.34b</td>
<td>1.26b</td>
</tr>
<tr>
<td>La Paloma</td>
<td>1.10bc</td>
<td>1.23bc</td>
<td>1.21bc</td>
</tr>
<tr>
<td>Tifway</td>
<td>1.05c</td>
<td>1.17c</td>
<td>1.08c</td>
</tr>
</tbody>
</table>
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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