Plant Root

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A GIS-based method for the analysis of digital rhizotron images

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Abstract: Quantification of belowground plant response via rhizotron root image analysis is difficult and time-consuming, yet a plant's root response is of great interest to many researchers. Here, we present an automated, time efficient method for examining digital rhizotron images. A total of 285 digital images (218 mm by 300 mm) were collected using a flatbed scanner from 16 rhizotron boxes from an experiment designed to evaluate the root response of Dalmatian toadflax, *Linaria dalmatica* (L.) Miller to herbivory by the Dalmatian toadflax stem mining weevil, *Mecinus janthinus* Germar, a widely used biological control agent. Images were quantified for root length and area using two methods: manually digitizing images using Root Measurement System (RMS) software, and semi-automated analysis using Feature Analyst™, an extension for a geographic information system. Feature Analyst length and area values were highly positively correlated with RMS area values, but were not correlated with RMS length measurements. The semi-automated Feature Analyst approach required one-eighth of the time required to analyze images using the manual RMS method. Feature Analyst for digital image analysis warrants more investigation, but appears to be a promising method for quantifying belowground plant characteristics.

Keywords: Dalmatian toadflax, Feature Analyst™, *Linaria dalmatica, Mecinus janthinus,* Root Measurement System

Abbreviations: RMS, Root Measurement System; FA, Feature Analyst; GIS, Geographic Information System(s); AFE, Automated Feature Extraction

Introduction

Although researchers often quantify aboveground responses of plants to varying growth conditions, environmental variables and experimental treatments, quantification of root responses is less common. Nevertheless, changes in belowground biomass may better indicate whole-plant responses to a number of phenomena, including herbivory, competition, application of herbicides, and altered soil nutrient and moisture regimes (Carlson and Donald 1988, Holland and Detling 1990, Callaway et al. 1999, Ziska et al. 2004, Collier et al. 2007, Ferrero-Serrano et al. 2008, Hodar et al. 2008, Thorne and Frank 2009). Additionally, quantification of root characteristics can be used to define the root distribution and architecture of plants in an effort to understand root function and plant-plant interactions (Reubens et al. 2007, Danjon and Reubens 2008).

Various forms of root system excavation are common for observation and quantification of root responses (Böhm 1979). Unfortunately, these methods are destructive and do not permit repeated measurement of individual root networks over time. The development of "glass wall methods," which have evolved into rhizotrons and minirhizotrons, allow non-destructive, *in situ* observation of changes in root systems (McMichael and Taylor 1987). The modern day rhizotron ranges in size from a small transparent container to a large underground trench lined with transparent walls (Muzik and Whitworth 1962, Böhm 1979, Polomski and Kuhn 2002). The minirhizotron consists of a small transparent tube that is inserted into the soil, usually at an angle, into which a fiber optic camera system or circular scanner is lowered for viewing and data collection (Meyer 1985, McMichael and Taylor 1987, Ingram and Leers 2001, Eizenberg et

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al. 2005).

Early techniques for enumeration of root length and root area involved tracing root images onto paper and measuring them, or counting root intersections with an overlaying grid system (Böhm 1979, McMichael and Taylor 1987). Overlaying grid and line intersection methods, established by Newman (1966) and modified by a number of authors (Reicosky et al. 1970, Marsh 1971, Rowse and Phillips 1974, Tennant 1975) are time consuming and lose accuracy as fine root density increases (Murphy and Smucker 1995). Digital capture of viewing planes through scanners and digital cameras have replaced manually drawn records, so methods for analyzing digital images have become more common (Meyer 1985, Dong et al. 2003).

Although rhizotron and minirhizotron systems are demonstrated technologies for observing root characteristics, quantification and analysis of rhizotron observations remain labor intensive and subject to error introduced by different users. These challenges are more pronounced when roots are analyzed against a soil background, which may impede root identification (due to similar coloration and interruption of roots by soil particles). Stahl and others (Stahl et al. 1995) found that 83% of the total variance in measuring fungal hyphal lengths on microscope slides was attributed to the people quantifying length. While this study was examining a different type of data collection, hyphae were quantified using a grid-intersect method comparable to some rhizotron image analysis methods. Nonetheless, the study demonstrated that observer subjectivity in ocular estimates of linear features could influence data quality.

Root Measurement System (RMS) (Ingram and Leers 2001), RooTracker (Duke University), WinRhizo Tron (Regent Instruments, Inc., Quebec Canada), ROOTEDGE (Kaspar and Ewing 1997), NIH Image (Kimura and Yamasaki 2001), CI-690 RootSnap! (CID Bio-Science, Inc., Camas, Washington) and numerous other root length estimating programs are software packages developed specifically for quantification of digital rhizotron and minirhizotron images. These software systems either require scanned images of washed roots, or require the operator to trace roots from a digital image using the computer mouse or a touch screen. Once complete, the software utilizes the digitized data to calculate measurements of total root length, number of roots, total root area, or other quantities of interest. While such systems are effective at collecting and organizing accurate root data, the data collection process is rather time consuming. In one study, Klatt (2006) reported that analyzing rhizotron images (measuring 21.8 cm by 30 cm) using RMS required as much as 40 minutes per image. Additionally, the individual user-centered orientation of these systems may introduce subjectivity and error associated with different techniques and skill level of the users.

Given the limitations of the conventional systems for root measurement described above, an alternative approach that is automated would be welcome by many researchers. The present paper demonstrates how geographic information systems (GIS) technology can be used for rhizotron image analysis. Geographic information systems are a collection of software and hardware systems developed to support the collection, manipulation, analysis, and presentation of spatial information (Longley et al. 2005). Though GIS is most typically used for analysis of "geographic" information, its analytic capabilities extend to analysis of spatial information at any scale. Given that rhizotron images can be considered spatial, we tested the efficacy of GIS-based image analysis technology, Feature Analyst™ (FA), for purposes of root analysis. The approach we tested requires the user to select a number of target features within an image. These features serve as "learning sets" for purposes of training the software to locate additional similar features within the image. The software system then extracts all like features and returns them as lines and polygons. O'Brien (2003) reported that the feature extraction capabilities were superior to manual feature extraction (digitizing) in conventional GIS for two reasons: (1) the automated approach classifies each pixel and therefore returns a more thorough and accurate extract; (2) the extraction results are more consistent among operators. The flexible settings within FA allow the user to extract features based on both shape and spectral characteristics, which are together important for extracting roots from a noisy soil background. Furthermore, GIS software, while proprietary, is widespread and many academic and research institutions possess licenses to operate GIS software. Feature Analyst software is available for trial periods at no cost, and extended licenses are available at reduced cost for academic uses.

This paper assesses the use of the GIS-based approach for quantification of plant root responses using a dataset from an experiment evaluating the effects of herbivory on an invasive plant (Dalmatian toadflax, *Linaria dalmatica* (L.) Miller) by an insect introduced for biological weed control (*Mecinus janthinus* Germar). Our main goal was to compare the GIS-based approach described above, FA, with manual digitization of rhizotron images using RMS. While we report on the results of the analysis with respect to the impact of herbivory by *Mecinus janthinus* on Dalmatian toadflax, those data are included to explore the methodology described herein, rather than to investigate the results of the biological control impact.

Materials and Methods

Experimental Setup

The study was conducted at the University of Wyoming Plant Sciences greenhouse facility in Laramie, Wyoming, U.S.A. A backhoe was used to excavate a trench measuring 460 cm long by 100 cm wide by 120 cm deep. The trench walls were reinforced with a plywood frame and the trench bottom was lined with a wooden plank foundation. An insulated cover over the trench minimized changes in soil temperatures within the open trenches. Rhizotron boxes (faces measuring 120 cm long by 15.2 cm wide and boxes measuring 30.5 cm deep) were made from 0.64-cm thick clear acrylic sheets. Approximately ten 1-cm diameter holes were drilled into the bottom of each rhizotron to allow adequate drainage. Boxes were filled with topsoil sieved to pass through a 1-cm wire screen, watered and allowed to settle. Boxes were then tilted 18° from vertical to allow roots to gravitropically grow down one side of the rhizotron (Muzik and Whitworth 1962) and placed in the trench to simulate natural growth conditions (below- and aboveground climates) at that location.

Dalmatian toadflax root crowns were dug from an infestation near Laramie in April, 2004. One root crown was planted per rhizotron and the plants were allowed to establish for one month. In early June 2004, *M. janthinus* were collected from a prior release site near Albany, Wyoming. Twenty adults were placed on each of eight randomly assigned rhizotrons and the other eight rhizotrons were used as untreated controls. This insect density has been shown to be sufficient for experiments examining the *L. dalmatica* – *M. janthinus* interaction (Breiter and Seastedt 2007, McClay and Hughes 2007). All 16 rhizotrons were individually covered with a fine mesh bags to prevent weevil movement between rhizotrons. Weevils were allowed to feed, mate, and oviposit for seven days before the mesh bags and all weevil adults were removed. Plants were watered as needed over the entire growing season, and plants were allowed to senesce in the fall.

Image Capture

Root images were obtained by placing a flatbed scanner (HP Scanjet 4670, Hewlett-Packard, Palo Alto, CA) onto the lower face of each container. Scans were captured every 305 mm down the rhizotron face, for a total of four scan depths per container. If no roots were visible, no scan was taken. Scanned images (JPEG format) measured 218 mm by 300 mm (1701 pixels by 2340 pixels). Root images were captured on eight dates throughout the growing season from May to October of 2004 and included a total of 285 images.

Image Processing

The rhizotron images used in this analysis were analyzed for root area and length using Root Measurement System, or RMS (see Ingram and Leers 2001). The same images were then evaluated using the semi-automated GIS-based method presented here, which requires a user to train the extraction algorithm based on a representative sample image. The image was brought into ArcMap™ Version 9.2 (ESRI, Redlands, CA) in raster format with red, green, and blue bands present and with no spatial reference information assigned. Using Feature Analyst™, or FA, (Overwatch Geospatial Systems, Sterling, VA), a set of features was identified to serve as the learning set from an arbitrarily selected image. The root image analysis process using FA is illustrated in Fig. 1. In order to capture as much variation in the learning set as possible, digitized training features were selected to capture variation within the target feature, including spectral variation, root branching patterns, and root segments throughout the entire image. The *Set up Learning* tool was then used to define the target features based on the identified learning set. For purposes of training the extraction algorithm, our specified extraction option was for narrow linear features. The *One-button Learning* tool was then used to train the extraction algorithm and to automatically extract the target features into a new polygon feature class.

Polygons with an area smaller than 500 pixels were then removed from the rhizotron images using the FA *Aggregate* tool. An aggregate size of 500 pixels was chosen after testing a variety of aggregate size settings. A setting of 500 was most effective in removing noise within the image while conserving root area. While removing small aggregates likely resulted in some root segments being removed, we felt it was the best attainable representation of root area due to the elimination of soil background noise.

The remaining polygons were then converted to lines using the FA *Convert to Lines* tool, which identifies the centerline of each polygon. In this step, the user can identify the following settings for line characteristics: (1) a minimum distance for which gaps in segments should be bridged; (2) a maximum length of segments and dangles that should be removed; (3) the maximum length of segments in intersections that should be repaired; and (4) a method for smoothing line features. The following settings were used for all images: gap jumping distance of 0 pixels; remove dangles with less than 50 pixels; re-work intersection segments with length less than 0 pixels; apply Bezier smoothing. These settings

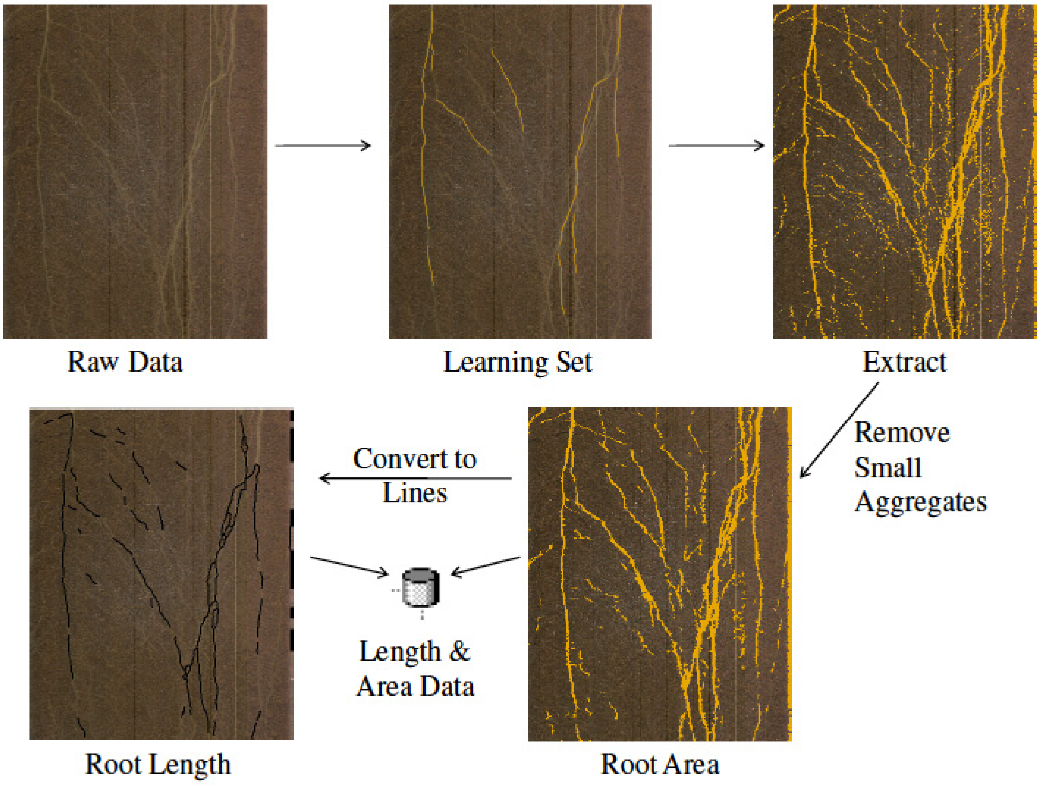

Fig. 1. Flow chart of root length and area extraction process using the Feature Analyst (FA) extension for ArcGIS.

produced lines that were most representative of the root centerlines for this image set.

It should be noted that the settings employed in this case were decided upon after much trial and error, wherein we visually discriminated against the results generated with each setting. While these settings were visually satisfactory for this image set (see Fig. 1), different image sets generated with different equipment and capturing different plant species will require the user to customize the settings accordingly.

One outcome of the initial training and extraction process is the generation of Automated Feature Extraction (AFE) files. These files are created with each process completed by FA and store settings required to help automate a workflow. The described process created four AFE files that could then be applied to all other images using the *Batch Classify* tool. Thus, one learning set and feature extraction procedure was applied to all images, creating consistency and limiting associated user subjectivity from image to image. The feature extraction process produced three shapefiles for each input image: two polygon shapefiles, (one with aggregates removed—the root area measurement), and one line shapefile (the root length measurement).

Examination of area and length files following extraction of all images revealed that some images had excessive amounts of area extracted due to clay film build-up on the surface of the rhizotron surface. These films were of similar spectral character to the roots in the learning set, and were thus extracted as root area. Images with clay films (51 images total) were then re-classified using a second learning set that was generated from a representative image within that set.

The root line and area features were exported to the native ArcGIS geodatabase format. Information stored in this format contains automatically calculated line feature length (pixels) and polygon feature area (square pixels). Area and length sums for each image were exported from each geodatabase to a spreadsheet. Length and area values were then converted from pixels to millimeters and square pixels to square millimeters respectively (each pixel measured 0.128 mm by 0.128 mm). The amount of time required to analyze images using the Feature Analyst software was noted throughout the process.

The RMS program and the FA approach calculate root area differently; the difference is inconsequential yet deserves explanation and is illustrated in Fig. 2. While the user is tracing root length in the RMS interface, he or she selects a root diameter that is representative of the root being traced. From the length and diameter data collected, the software calculates the surface area (in mm^2) of the root as if it were a cylinder. Conversely, the FA system calculates a surface area (in mm^2) directly from the count and

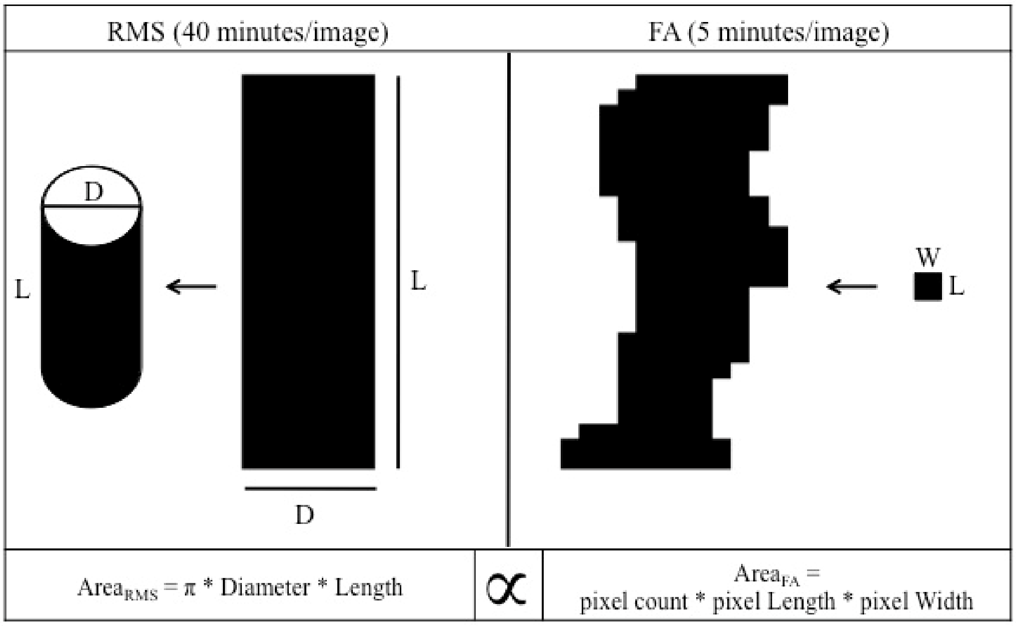

Fig. 2. Conceptual diagram of methodological approaches of Root Measurement System (RMS) and Feature Analyst (FA) software systems. With the RMS method, surface area of a cylinder is calculated based on length and diameter dimensions provided by the user while tracing roots. The FA method classifies root material based on spectral characteristic (directed by the user-created learning set), and then provides the summation of all root area (representative of root cross sectional area). The area calculations are directly proportional by a factor of π ($Area_{RMS}$ / π = $Area_{FA}$). The average time for individual image analysis with each method is listed and represents total tracing time (RMS) or total time for developing a training set, extracting, and data manipulation (FA).

dimensions of pixels classified as root by the program. Cross sectional area (represented by the FA values) is directly proportional, by a factor of π, to the cylindrical surface area (represented by the RMS values). We converted the surface area values generated with RMS to represent the cross-sectional area measurement for purposes of comparison. The conversion did not change any of the statistical analysis results. Therefore, we present the area values as they are obtained from each software program, recognizing that the area values are calculated differently, but the difference is relative and does not influence analysis results or conclusions.

Data Analysis

Data extraction using RMS is considered to be a standard practice for root analysis (Ingram and Leers 2001, Kuchenbuch and Ingram 2002) and thus serve as a benchmark to support validation of the FA method. We tested the accuracy of the FA data relative to the RMS data as well as the consistency of the conclusions reached via each analytical approach.

Data generated with both methods did not adhere to the normality assumption, as indicated by Shapiro-Wilk tests and Q-Q plots. Of the image set, many images had low root length and area, and few images had high root length and area, so data assumed an exponential distribution. Transformations of the data were unsuccessful in alleviating this problem; therefore, all data were subject to nonparametric statistical methods. Data were examined with Spearman rank order correlations (Spearman's rho) for detection of relationships between (RMS length – FA length; RMS area – FA area) and within (RMS length – RMS area; FA length – FA area) root analysis methods. Pair-wise mean comparisons using the Mann Whitney U test examined length and area values within (*Mecinus* – control) and between (RMS – FA) root analysis methods. Again, these comparisons were performed to explore the consistency of conclusions reached via each approach, rather than to explore the effect of the biological control agent. All data were analyzed using R software for Mac OS X version 2.9.2 (www.R-project.org).

Results

Root length values generated with the two analysis methods did not exhibit a strong relationship ($\rho(283) = 0.293$, $p < 0.001$). A scatterplot of the length values (Fig. 3A) shows a great deal of variation, and the FA values are smaller in magnitude. When root area values generated by the two methods were compared, they had a strong positive relationship ($\rho(283) = 0.974$,

$p < 0.001$). Further, the scatterplot (Fig. 3B) shows a tight relationship between the values.

After examining correlations between the two methods, we evaluated each method for internal consistency. Scatterplots and Spearman rho coefficients relating length values to area values *within* each analysis method were computed. When RMS length and RMS area were compared, the relationship was weak though statistically significant ($\rho(283) = 0.294$, $p < 0.001$) (Fig. 3C). The FA length and area values had a strongly positive and tight relationship ($\rho(283) = 0.972$, $p < 0.001$) (Fig. 3D). Correlations were examined using the individual images, regardless of plant identity, scan date, or depth; however, when we broke down the dataset according to plant identity, scan date, and depth, these relationships remain. Therefore, we are confident that these results are consistent across the entire image set.

The large degree of variability associated with RMS length data prompted us to more closely examine variability within the datasets. Fig. 4 presents box plots with whiskers for length, area, and length-to-area ratio for each image analysis method. Root length, as measured by RMS was larger in magnitude and variability than FA root length (Fig. 4A). The opposite trend was observed with root area, where FA area was larger in magnitude and variability than RMS area (Fig. 4B). Because of these differences, the RMS method produced large and highly variable length to area ratios, whereas the FA method produced small ratios within a narrow range of values (Fig. 4C).

When total length and area (per plant) were separated according to sampling date, there were few differences in root length and root area when tested for effects of treatment (attack by *Mecinus* or control) (Fig. 5). The RMS method indicated that on 1 July and 28 July total root length of control plants was greater than those treated with *Mecinus* ($U(N_M = 8, N_C = 6) = 5$, $p = 0.013$; $U(N_M = 8, N_C = 6) = 8$, $p = 0.04$ respectively) (Fig. 5A). However the RMS method also

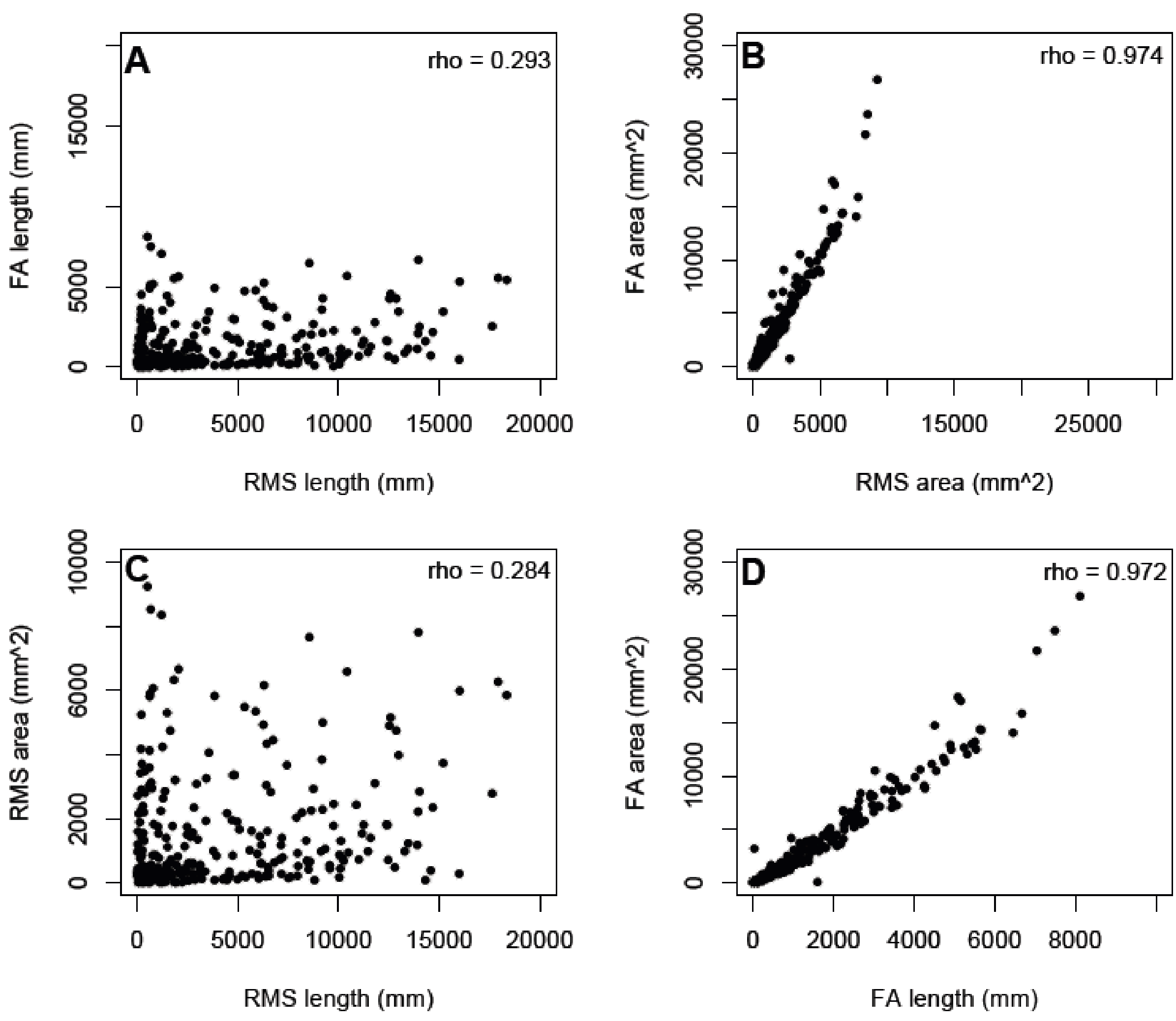

Fig. 3. Scatterplots with Spearman rank order correlation coefficients (rho) for relationships between Root Measurement System (RMS) and Feature Analyst (FA) measures of root length and root area.

indicated a reduction of root length of control plants during the last two months of the study, resulting in a significant difference between treatments on 25 October (U(N_M = 8, N_C = 6) = 40, p = 0.04). The RMS length data appear to be inconsistent with RMS area and both FA measurements, which consistently indicated greater root length and area of control plants compared with damaged plants on 10 August (U(N_M = 8, N_C = 6) = 4, p = 0.008 for all) (Fig. 5B – 5D). The fact that we did not observe large *Mecinus* treatment effects limits our ability to rigorously test the consistency in conclusions gleaned from the two analysis methods; however, the data trends yielded by the FA method appear to align well with those obtained from RMS area data.

The values obtained by each method exhibit clear differences in magnitude. Pair wise mean comparisons of length and area values indicated that RMS length values are consistently and significantly larger than FA length values (U(N_M = N_C = 112) = 8016, p < 0.001) (Fig. 3A), whereas FA area values are consistently and significantly larger than RMS area values (U(N_M = N_C = 112) = 487, p = 0.003) (Fig. 3B). These differences exist regardless of scan date or treatment.

Discussion

Rhizotron studies of the impacts of herbivory (or other variables) on root growth implicitly require intensive, time-consuming analysis of rhizotron images. Software packages specifically designed for quantification of these images, such as RMS require the operator to manually digitize root paths. Our main goal was to assess the use of an alternative approach using GIS-based feature identification methods, particularly with respect to accuracy, automation, and time efficiency.

Our results indicate that the GIS-based method, FA, produced root length and area values that correlate well with area values generated by RMS; however, they did not correlate with RMS length values. We believe that one explanation for the variability observed when comparing RMS length with both FA and RMS area measures relates to the differences in how each method generates the data. The RMS calculation of root length is generated as the user traces visible roots on the image; area is then calculated based on diameter class selected by the user. Conversely, the GIS-based method first extracts root areas based on pixel spectral characteristics, and then converts individual areas to lines using algorithms internal to the software. This difference, illustrated in Fig. 2, may account for the tight relationship observed between FA length and area values, but lacking between RMS length and area.

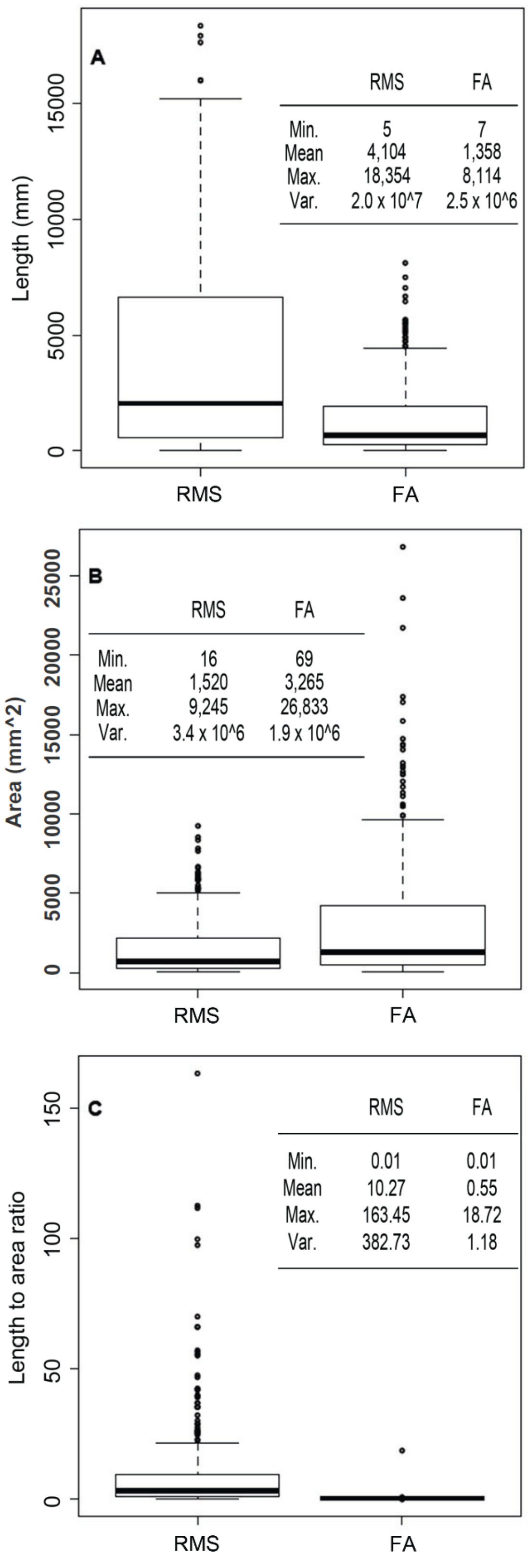

Fig. 4. Box-and-whisker plots and descriptive statistics for (A) root length measures; (B) root area measures; and (C) root length-to-area ratios for Root Measurement System (RMS) and Feature Analyst (FA) analysis methods.

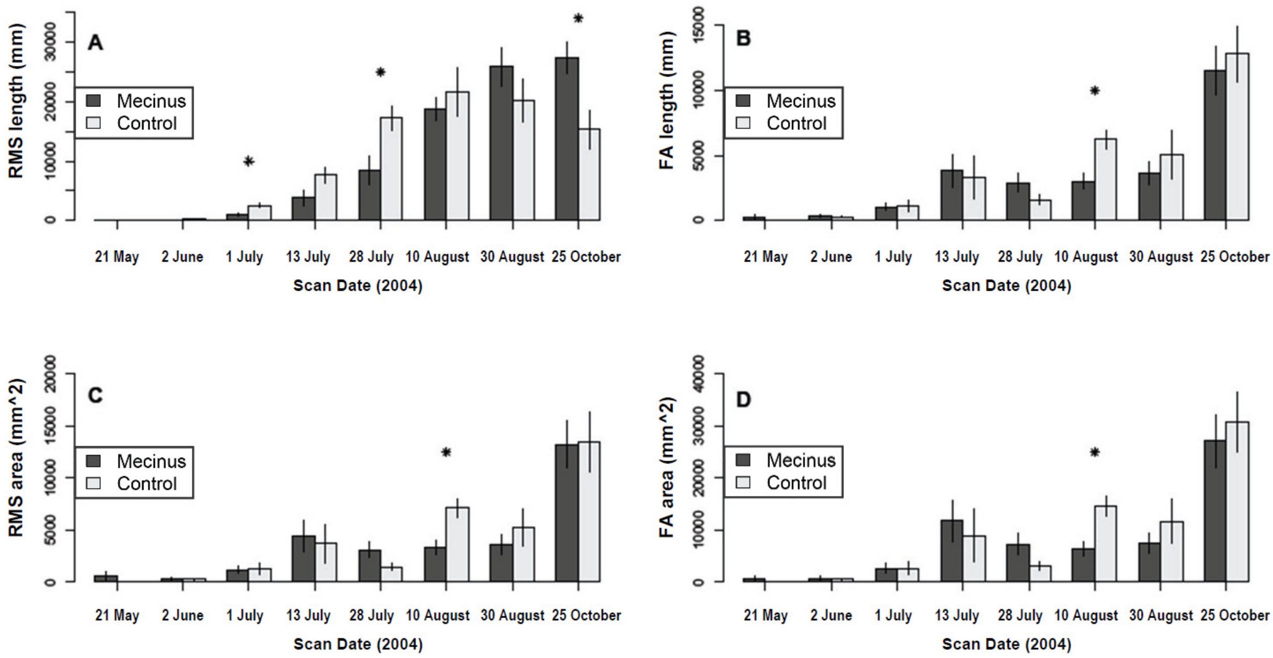

Fig. 5. Bar graphs illustrating root length and area measurements for Root Measurement System (RMS) and Feature Analyst (FA) analysis methods, according to rhizotron scan date and exposure to *Mecinus janthinus* (Mecinus) or exclusion of *M. janthinus* (Control). Error bars represent ± mean standard error.

Comparison of the two methods also shows that FA root length values produced from an image were typically less than half of the length of RMS root lengths (Fig. 4A). Again, we believe that this difference is a result of the user-centered versus software-generated approaches of the software systems. When a user is manually determining root material (with RMS), he or she may be more likely to interpolate gaps in roots that are clearly continuous, but are interrupted by soil debris, clay films, etc. Conversely, the automated feature extraction (with FA) would not include such gaps based on spectral characteristic. Additionally, the settings employed with the FA method for removing noise and converting to lines likely eliminated segments of roots that would be included with the RMS method. Fig. 1 clearly shows that the lines generated with FA are an underestimation of roots for that image. Because the settings are flexible, we believe that length estimation can be improved within the FA methodology to better represent roots and differentiate between root and non-root material.

While length estimates obtained by the two methods were not correlated, we found that FA provided accurate measures of root area when compared with the RMS method. However, unlike root length estimates, values for root area generated from FA were larger than those generated by RMS, not smaller (Fig. 4B). The larger values estimated by FA may have been due to inclusion of non-root background noise. For instance, in Fig. 1, the right-hand side of the image has area classified as root, which is likely a blemish or glare on the scanner surface. Additionally, the FA method has the ability to classify irregularly shaped root area, rather than estimate roots as a perfect cylinder (see Fig. 2), which may also contribute to larger area estimates by FA.

When we examined the effect of treatment on root length and root area, our overall conclusion with respect to treatment was consistent between the two methods; the belowground growth of plants did not respond to herbivory over the course of the study. Yet, we did find inconsistencies between the measurement methods on specific dates. These inconsistencies are likely to reflect the differences in how root length and root area are measured by the two methods, and the factors that affected these estimates that have already been discussed.

Overall, our results suggest that GIS-based FA analysis of rhizotron root images may offer a useful alternative to manual digitization of root images with root analysis software packages, such as RMS, although there are some potential trade-offs in addition to strengths. First, FA did not yield good estimates of root length when compared to the accepted RMS method. Conversely, estimation of root area appeared to be more robust, and gave good, albeit overestimated, root area values. Second, there is the potential for subjectivity with FA in establishing the learning set. Nevertheless, each image is classified according to the same parameters, which eliminates error within a dataset associated with user fatigue, unsteady tracing, or multiple users, all issues associated with the other method. Lastly, a major strength of FA software was that images analyzed with FA required one-eighth of the amount of time required for analysis with RMS software. As mentioned previously, Klatt (2006) reported that analysis of rhizotron images using RMS required as much as 40 minutes per image. We found that analyzing images of the same size with FA required less than five minutes per image. This estimate includes the time required to create the learning set, export data to spreadsheets, and convert measurement units from pixels to millimeters. Given the efficiency of this method, all images analyzed with FA were completed in one day. The FA software has a user-friendly interface, which requires minimal learning if the user has a basic knowledge of the supporting geographic information system. Furthermore, because the workflow is automated, we were able to continue working on alternative tasks during the extraction process.

In conclusion, we feel that GIS-based analysis of digital rhizotron images for obtaining root length and area data, as described here, warrants further investigation into its validity. The method is automated and time efficient, and should be examined in multiple soil types, with multiple types of rooting structures and patterns. The method should also be compared with other available additional accepted software programs and destructive sampling for its level of accuracy and precision, and for exploring measurement bias (small length values and large area values) should also be investigated. If this analysis tool can be more rigorously tested and accepted, its application would be diverse and useful. The ability of FA to classify pixels based on spectral characteristics could potentially allow researchers to differentiate between roots of multiple plant species, or to identify and quantify root death and turnover. Image analysis would not need to be limited to rhizotron and root images and could be expanded to classification and quantification of digital images of microscope slides, leaf surfaces, and field plots. In short, the GIS-based method for image analysis appears promising and should be further investigated for its validation and use on rhizotron images.

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