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Digital Acquisition and Measurement of Peanut Root Minirhizotron Images

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ABSTRACT

Video, photo, or in situ image capture and analysis from minirhizotrons is time-consuming. Computerized root image analysis is expensive and subject to large background error. This paper describes a fast, simple, and accurate method for (i) capturing minirhizotron images through low-cost hardware upgrade of existing minirhizotron equipment, and (ii) processing digital images using low-cost software. This system was developed to monitor root distribution in peanut (*Arachis hypogaea* L.). Digital images were recorded from a minirhizotron camera directly to a laptop computer hard drive instead of using videotape. This reduced image acquisition time by 36% and eliminated problems associated with manipulating video players. The image analysis system uses a mouse pointer to trace root length and diameter on a computer screen and calculates the distance traversed by the mouse pointer. A paired *t*-test on accuracy using wires of known length showed no significant difference ($P = 0.10$) between actual and measured lengths.

Images from minirhizotrons are often collected on tape with a video recorder, then transferred to a computer using a video capture card (e.g., Franco and Abrisqueta, 1997; Goins and Russelle, 1996; Johnson and Meyer, 1998; McLean et al., 1992; Smit and Zuin, 1996). Videotape images may either be translated into digital pictures to quantify root measurements with a computer (Smucker et al., 1987), traced with a linear probe on a monitor (Beyrouty et al., 1987), or counted with a grid on a monitor or photograph (Vos and Groenwold, 1987). Bypassing translation of video to digital image through direct storage of images in their final digital form will greatly reduce time needed for minirhizotron image acquisition. Transfer from moving video images to still digital images requires as much time as does recording video images from a minirhizotron and tracking successive images to capture or trace. Further, constant starting and pausing of a video player strains both tape and recorder, rapidly degrading recorded image quality. Some new minirhizotron models store images directly from a camera to a laptop computer hard drive (http://www.bartztechnology.com/ICAP.htm). Older equipment can be upgraded using low-cost, off-the-shelf hardware and freeware or shareware software. Our first objective is to describe such an upgrade.

Minirhizotron usefulness is limited by difficulty, complexity, and cost of image analysis (Hendrick and Pregitzer, 1996). Available PC-based programs need to be adapted to requirements of current research (Andren et al., 1996; Majdi and Nylund, 1996), or new programs must be developed (Smucker et al., 1987). Development of image analysis programs requires large material and time investments and can significantly delay a research program. Choice of an existing image processing software is limited by cost, complexity, computing hardware requirements, image storage format required, versatility, speed, accuracy, and reliability. Our second objective is to describe a fast, simple, accurate, and inexpensive image analysis system to measure peanut root lengths from minirhizotron images.

MATERIALS AND METHODS

Minirhizotron Image Acquisition System Upgrade

Root images were viewed at the top of 5-cm i.d. minirhizotron tubes installed horizontally at six depths in soil-filled barrels. Images were collected at 5-cm intervals along the tube, starting 5 cm from the edge of the drum to the center of the tube. Images were collected at 14-d intervals from planting to harvest. Three digital images of a 1- by 1-mm grid placed on top of a spare minirhizotron tube were taken before taking a set of root images. The first two images were discarded and were only taken as test shots to condition image set and recording after the system was powered on. The third grid image was used in calibrating the software for measuring root lengths in all images recorded up to the time the camera control box was powered off.

Initially, data were recorded using a minirhizotron camera (BTC-100X[1], Bartz Technology, Santa Barbara, CA) and VCR (Model CCD-TRV70, Sony Electronics, Park Ridge, NJ). A 3-s pause after positioning the camera was sufficient to collect a video image that could be captured by the desktop computer. Images from videotapes were transferred to a desktop PC

[1]Use of a specific brand name does not imply endorsement by the University of Georgia.

Abbreviations: BMP, bitmap graphics file; GIF, graphics interchange format; JPEG, joint photographic expert group; NTSC, National Television Standards Committee; PAL, phase alternating line; PCI, peripheral component interconnect; PP-VCD, parallel port video capture device; SECAM, sequential color and memory; TIFF, tagged image file format; VHS, video home system.

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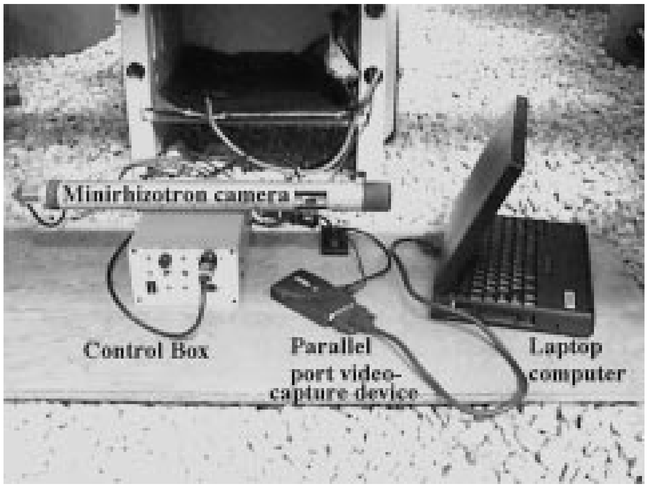

Fig. 1. Minirhizotron image acquisition system.

using a peripheral component interconnect (PCI) video capture card on a desktop computer. A series of 20 batch image collections, with each batch consisting of five images, was taken using this system. The time required for each batch reading was noted.

A data acquisition system upgrade was made to capture images directly from a minirhizotron camera to a laptop computer hard drive. The basic components were (i) existing minirhizotron camera; (ii) parallel port video capture device (PP-VCD); and (iii) laptop computer (Fig. 1, Table 1). The video output signal from the minirhizotron camera was used as input to the PP-VCD. The PP-VCD was attached to the parallel or printer port of the laptop computer. Power supply was derived through a pass-through cable plug connection to either the keyboard or mouse port of the laptop. The PP-VCD is compliant with the standard protocol for communications between software and image acquisition devices, also called Twain protocol. The PP-VCD has options for saving images in either joint photographic expert group (JPEG), tagged image file format (TIFF), or bitmap graphics file (BMP) formats at sizes ranging from 320- by 240- to 1600- by 1200-pixel resolutions. The Photoimp Version 2.5 software used for capturing 640- by 480-pixel color images in JPEG, BMP, or TIFF formats also comes with the PP-VCD. This software also allows a compression ratio to be defined for the JPEG output. Twenty batches of direct computer image capture were taken, with each batch consisting of five images. The time required for each batch reading was recorded. Times required for digital image collection before and after the upgrade were compared.

Minirhizotron Root Image Processing

Root lengths and diameters from digital images were measured by tracing roots along the computer screen using the mouse pointer. All software used are shown in Table 1. A freeware program, Mouse-O-Meter Version 1.1, was used to monitor the distance traversed by the mouse pointer. This program is available on the Internet at http://hotfiles.zdnet.com/cgi-bin/texis/swlib/hotfiles/info.html?fcode=000OVK. Mouse-O-Meter runs on a Windows 95/98 operating system and requires about 217 KB memory. A shareware graphics program, Polyview 3.20.1, also available via the Internet (http://www.polybytes.com), was used to display the image, while Mouse-O-Meter logs distance traversed in each root image. Polyview shows the pixel coordinates of the mouse pointer at any location in an image, from which endpoint coordinates of roots were recorded to track their growth and position in subsequent readings. All readings were recorded separately, either in a spreadsheet program or manually using data sheets.

Calibration is a two-step process. First, Mouse-O-Meter software is calibrated to measure real physical distance on the computer screen. Then a calibration factor is calculated based on measured physical screen distance and known actual line length within the calibration grid image The first calibration step configures the software for monitor size, chooses a unit of measurement, and adjusts the net display size to match actual Mouse-O-Meter readings. Available monitor configurations are 36, 38, or 43 cm, for 14-, 15-, or 17-inch monitors, respectively. We used a 36-cm monitor configuration. Adjusting the mouse pointer motion property to the slow setting is advisable for greater accuracy in display tracing. This adjustment can be made in the Windows Control Panel's Mouse Properties. Net display size is adjusted by running the mouse pointer from the top left to top right corners of the screen and noting distance reading given by Mouse-O-Meter. Distances registered by Mouse-O-Meter are cumulative, like an odometer, and the difference between the final and initial measurement is the net distance traversed by the mouse pointer. A confirmation Mouse-O-Meter measurement should be made from the bottom left to bottom right corners of the screen and similar distance reading should be logged as described above. A constant reading of 261 mm was logged regardless of the speed of mouse movement, both in the top and bottom horizontal runs. Similar runs were done from the top left to bottom left, and top right to bottom right corners of the screen. Readings of 196 mm were logged in each run. A rectangular piece of paper 261 by 196 mm was cut and placed flat on the screen. Using the screen video control knobs, the net image display was adjusted to measure 261 by 196 mm. This step sets Mouse-O-Meter to measure real physical distance traversed by the mouse on the computer screen.

The second calibration step was done using Polyview graphics program to display the 1- by 1-mm calibration grid image, and tracing vertical and horizontal lines with known lengths in the image. Any graphics image program can be used for displaying root images provided actual pixel image size is displayed on screen; that is, by setting zoom options to 1:1. A

Table 1. Equipment and software used for minirhizotron image capture and analysis.

Device/program	Model/function	Upgrade costs
	Hardware	
Minirhizotron camera	Bartz BTZ-100X camera	Existing camera
Parallel port video capture device	Zipshot	$99
Laptop personal computer	IBM ThinkPad Type 2635AU	Existing nondedicated laptop and used with other research processes
	Software	
Photoimp	Digital image capture program	Free with Zipshot parallel port video capture device
Polyview	Graphics image display and filing system	Shareware; $25
Mouse-O-Meter	Screen distance mouse tracking software	Freeware
Total cost		$124

calibration factor was computed by dividing the actual known distance in the image grid by the physical distance on screen measured by Mouse-O-Meter. The quotient is the calibration factor, which when multiplied by Mouse-O-Meter readings in root images, converts them to actual root length.

A test of the measurement system after calibration was done by using 20-mm-long, 0.26-mm-o.d. strands of wire. Twenty wire strands were placed at random orientation on a graph paper with 1- by 1-mm grid, and wrapped around a minirhizotron tube. Images were taken using the minirhizotron camera, and lengths were measured as described above.

RESULTS AND DISCUSSION

Minirhizotron Image Acquisition System Upgrade

For video images, it took an average of 9.4 s (SE = 0.4) to record an image and move the camera to the next location; plus 7.9 s (SE = 0.6) to capture an image from VHS tapes to digital images with a desktop PC. A total of 17.3 s are needed to record and capture each digital image using this process. The use of a parallel port video capture device required 11.0 s (SE = 0.6) per image for digital image acquisition on a laptop computer, 6 s less than using video recorder and video image capture from VHS tapes. This shortens image collection and storage time for each sample day by at least 1.5 h for our experiment, with at least 900 images measured on each sample date. The resulting images taken were observed to be clearer than those captured from VHS tapes, and problems associated with manipulating a video player, timing of screen capture, and wear and tear of the tape medium are eliminated. In both systems, image frames without roots can easily be ignored, as done in our earlier readings using video systems. The ease with which images are collected using a PP-VCD enables quick storage of all pictures, and comparisons can be made as roots develop over time in a series of images taken from the same tube location.

Existing hardware can be upgraded simply using an off-the-shelf PP-VCD that attaches between a minirhizotron camera and a laptop computer. The PP-VCD we used cost less than $100. Several Internet sites offer PP-VCD (e.g., http://www.play.com; www.arcsoft.com). Factors to consider in selecting a PP-VCD are signal output of the existing minirhizotron video camera, available software to direct the image capture, speed of the capture process, output image pixel size, and storage format. Video signal in either National Television Standards Committee (NTSC), phase alternating line (PAL), or sequential color and memory (SECAM) format from the minirhizotron camera must match the configuration of the PP-VCD.

Capture speed is important when as many as 1000 images are collected on each sample date. Capture speed and hard disk storage requirements are largely a function of file size and image format (BMP, JPEG, TIFF, or GIF). The JPEG format has the smallest file size and fastest recording time on a computer hard drive. Although JPEG has a lossy compression scheme, this is not a disadvantage when roots are traced using a mouse meter program. Image capture software should have built-in options for selecting a variety of image sizes and storage formats. This will allow flexibility in the choice of image analysis software. Usually, PP-VCDs include the cables and software necessary to capture images from a minirhizotron camera, and different file storage format options are available. A compact disc writer is highly recommended for storing large numbers of images.

The use of a laptop computer instead of a VCR requires a small cart-platform holder for the laptop, preferably with a continuous power supply from a portable generator. A black plastic canopy hood placed on top of the laptop monitor provides a clear image preview. Although this setup limits the portability of the system and requires a lightweight mobile cart, measurements done in cart-accessible areas reduced heavy load strain on the operator, and greatly reduced time required for data acquisition.

Minirhizotron Root Image Processing

Average calibration factors of 0.09950 (vertical) and 0.09537 (horizontal) mm actual root length per mm physical screen distance were obtained from 20 readings in each direction. Differences in vertical and horizontal calibration factors suggest a slight distortion as the image is converted from analog video signal to a digital image. This distortion is similar to that observed by Zoon and van Tienderen (1990). If there are no distortions and vertical and horizontal calibrations are equal, the use of the calibration factor as multiplier for the mouse meter readings will directly give the actual root length. If there is image distortion, root orientation must be noted when measuring roots. Screen distances measured for horizontally oriented roots are multiplied by a factor of 0.0995 mm mm^{-1}, and 0.09537 mm mm^{-1} for vertical roots. The actual diagonal root measurement can be computed from given pairs of endpoint pixel root coordinates, x_1, y_1 and x_2, y_2; vertical and horizontal correction factors, VCF and HCF; and the diagonal mouse meter distance reading, M. The actual root measure can thus be computed as

$$\left\{\left[M \times \sin\left(\arctan\frac{y_2 - y_1}{x_2 - x_1}\right) \times \mathrm{VCF}\right]^2 + \left[M \times \cos\left(\arctan\frac{y_2 - y_1}{x_2 - x_1}\right) \times \mathrm{HCF}\right]^2\right\}^{1/2}$$

The measurement system cannot be used with S-shaped or coiled roots if there is horizontal and vertical distortion in the digital image. No coiled or S-shaped roots were found within any minirhizotron image frame. Direction orientations tend to be distinct for an individual root in an image, and the above factors are easily applied.

User training can be done by measuring vertical, horizontal, and diagonal lines with known lengths, then comparing actual with measured readings. Such practice measurements are recommended before each session to ensure uniformity of movement by the operator and to check the readings. Also, the operator must keep track of roots already measured. An average measurement reading of 19.9 mm was obtained on 20-mm wires, with an error of 0.5%. Paired *t*-test analysis using the PROC MEANS T PRT procedure of SAS (SAS Inst., 1990) showed that the mean difference between the measured length and corresponding true value of wire length was not significantly different from zero ($P = 0.10$). A single root seldom has more than 20 mm length visible in one 14- by 18-mm frame captured by the minirhizotron camera. Shorter roots are more easily traced and less prone to error than longer roots because short roots can be traced without moving the whole hand. Digital images without vertical and horizontal distortion should result in more accurate readings with a single calibration factor on the mouse meter. Stretching the screen image in the horizontal or vertical axis may compensate for distortion, but this process may be too cumbersome for a large number of images. Calibration factors are simpler to use, and numeric processing can be done by standard statistics, spreadsheet, or other analysis software.

Processing minirhizotron images in order to measure root length and root diameter is difficult with automated image analyzing software because of the great background noise (Andren et al., 1996; Zoon and van Tienderen, 1990). Large computing resources are often needed for image processing, and software used often requires a large image file storage format such as TIFF (Kaspar and Ewing, 1997) and BMP. Automatic image analysis can be done most easily when the background contrasts sharply with the root image. In a minirhizotron image of roots growing in soil, however, extraneous spots and other noise occur even with several steps of filtering and contrast enhancement (Andren et al., 1996). Also, earthworms, nematodes, and rootlike soil organic structures can only be identified by a trained operator doing the specific research. The use of a semiautomatic system of root image measurement like the Mouse-O-Meter provides a more specific measure when large background noise and many rootlike structures are present in the image.

In summary, the use of a PP-VCD to replace the video recording and capture of images from tapes resulted in higher-quality digital root images from minirhizotrons, and reduced time needed for image capture by 36%. Existing minirhizotron systems can be easily upgraded at low cost to directly capture digital images and eliminate problems involved in the use of video recorders and tapes. Semiautomatic root length measurements can be done on minirhizotron images by tracing roots on screen with the mouse pointer with freeware programs like Mouse-O-Meter. This simple method of measuring root lengths is versatile, fast, and accurate. Accuracy can be enhanced with proper calibration and verification at each batch of readings, and through initial training of the operator on the use of the mouse pointer tracking software. A mouse meter root tracking program based on this setup is currently being developed to automate calibration and data recording.

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