## V. Comparison of Digital Image Analysis Software for Determining Virginia Buttonweed (*Diodia virginiana*) Regrowth as Affected by Herbicide Treatment

**Abstract**: Digital image analysis (DIA) has been successfully used to quantify turfgrass coverage during establishment. A field experiment was initiated during spring of 2001 to determine if DIA could be used to monitor individual Virginia buttonweed plant response to herbicide treatments. Digital images were collected 19, 30, 88, and 115 days after initial treatment. Images were collected with a Nikon 990 digital camera precisely positioned 1 m above each plant. ERDAS IMAGINE 8.5 and SIGMA SCAN 5.0 software packages were then used to analyze the images for percent ground coverage of Virginia buttonweed in a 1-$m^2$ area over time. The numerical values were assigned from pixel association by each program. There were 1.71 million pixels captured in each 1-$m^2$ image. Percent coverage was computed by dividing the number of pixels associated with Virginia buttonweed by the total number of pixels present. ERDAS IMAGINE 8.5 consistently reported more plant material than SIGMA SCAN 5.0 and the magnitude of difference varied by date of image collection. Except for date 3 images, both software programs produced significantly different numerical values. However the image values always had a linear relationship. Both of these programs could be used to precisely measure the percent of Virginia buttonweed in a set area in the presence of a highly contrastable background.

**Nomenclature:** Virginia buttonweed, *Diodia virginiana* L. #[1] DIQVI

**Additional Index Words:** Virginia buttonweed regrowth.

**Abbreviations:** DIA, digital image analysis; DAIT, days after initial treatment; MAT, months after treatment.

## INTRODUCTION

Visual evaluation parameters of turfgrass quality are routinely used to assess performance characteristics of turfgrass cultivars in field plantings. Rating systems are commonly employed by researchers to measure phenotypic variations in color, density, and uniformity of turfgrass species. Individuals perceive plant materials differently and thus have altered judgments on color and uniformity of turfgrasses (Gnegy 1991). Horst et al. (1983) studied 10 evaluators for their ability to quantify turfgrass quality and color. They determined that the variability within evaluators was equal or greater that the variation among turfgrass cultivars evaluated.

Many kinds of information are available to our eyes in a qualitative sense but require large investments of time and instrumentation to confirm quantitatively (Ewing and Horton 1999). In some early quest for quantitative turf evaluations, Madison and Anderson (1963) used a chlorophyll index to determine the amount of turf present in a specified area and were able to detect differences in fertilizer regimes.

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[1] Letters following this symbol are a WSSA approved computer code from *Composite List of Weeds*, Revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

Fenstermaker-Shaulis et al. (1997) used a multispectral scanner to evaluate tall fescue (*Festuca arundinacea* Schreb.) response to drought stress. Evapotranspiration, canopy temperatures, biomass, and tissue moisture content were measured to coincide with the remote sensing data. They concluded that high spatial resolution (1 m) multispectral data could provide accurate values to assess turfgrass health on a microscale within large irrigated areas.

Image analysis is a technique that divides analogue pictures into a grid where each individual cell represents a pixel. Each pixel has a numerical value that measures a particular color. It is the task of digital analysis software to group and differentiate these various subgroups of color into areas of interest that will provide valuable information about plant health or ground cover.

Salinity effects on St. Augustinegrass (*Sternotaphrum secundatum* [Walt.] Kuntze) cultivars were evaluated using image analysis to quantify stress responses (Meyer et al. 1989). A positive linear correlation was obtained between shoot area as determined by image analysis versus dry weight measurements. They concluded that the use of image analysis maximized assessment of turfgrass stress responses and quantification.

Richardson et al. (2001) utilized SIGMA SCAN PRO v. 5.0 to evaluate percent turfgrass cover in a zoysiagrass (*Zoysia japonica* Steud.) grow-in study. Digital analysis and visual assessment of plots over time provided the same chronological rank of plots with respect to percent turfgrass cover. They concluded that digital image analysis proved to be an effective and efficient method for evaluating the amount of turfgrass in a defined area.

There is a need in turfgrass research to quantify certain measurements, which could also be quantitatively interpreted (Madison and Anderson 1963). Currently no published reports on the use of digital imagery to quantify weed or turfgrass response to herbicides are available. Therefore, this study was conducted to investigate the use of digital image analysis for determining Virginia buttonweed response to selected herbicides.

## MATERIALS AND METHODS

**Experimental procedure.** Virginia buttonweed seed was collected from the Auburn University Turfgrass Research Unit in the fall 2000 and stored in a controlled environment (7 C and 42% relative humidity) for 3 months. Seeds were sown March 2001 into growth media where they were allowed to go through wetting and drying cycles to stimulate germination. Once individual seedlings had reached a height of 3 to 5 cm, they were transplanted into 1-L styrofoam cups containing a 90:10 v/v mixture of sand:peat. Transplants were grown for 55 days in a greenhouse (21-32 C) and watered four times daily. Biweekly, each cup received 50 ml of a fertilizer solution containing 4 ml /L of 20-10-20. Previous observations of the growth of Virginia buttonweed seedlings in the above greenhouse environment proved it to have functional perennial rhizome structures 6 weeks after emergence.

The field site was located on a Marvin sandy loam soil (fine-loamy, kaolinitic, thermic Typic Kanhapludults) consisting of 1.2% organic matter and pH of 6.0. During the previous fall, the site was fumigated with metam-sodium (Vapam®) at 123 liters of product per hectare and seeded to perennial ryegrass (*Lolium perenne* L.) to stabilize the

soil. Individual 1.2- by 6-m plots spaced on 1.2-m centers were treated with glufosinate at 0.84 kg ai/ha to provide a plant-free environment for transplanting the greenhouse-grown Virginia buttonweed plants.

Three holes with an in-row spacing of 1.5 m were created with a putting green cup cutter in individual plots that contained the desiccated perennial ryegrass. Virginia buttonweed plants were removed from the styrofoam cups and the entire contents of one cup placed into a hole. Each plant was then fertilized with 100 ml of the previously described fertilizer solution. In the absence of natural rainfall, supplemental irrigation was applied to achieve at least 6.4 mm per week. Plants were allowed to grow for 40 days under these conditions before herbicide treatments were applied. With exception of the Virginia buttonweed plants, plots were maintained weed free throughout the experiment.

Treatments were arranged in a four herbicide by two application factorial and placed in a randomized complete block design with two replications. Appropriate non-treated controls were also included in each block. Herbicide treatments were applied 9 July, 2001 with a $CO_2$ backpack sprayer calibrated to deliver 280 L/ha at 213 Kpa. Cohort DC®[2] adjuvant was used at a rate of 1.5 g/L of spray solution. Virginia buttonweed response was evaluated 19 and 30 days after initial treatment (DAIT) and approximately monthly thereafter. The above-ground portions of Virginia buttonweed plants within treatments receiving a single application were removed immediately following the 30

[2] Cohort DC is a proprietary blend of polyethoxylated hydroxyl alkyl surfactants, encapsulated in organic nitrogen. Helena Chemical Company 225 Schilling Blvd Collierville, TN 38017.

DAIT images. The area around the individual removed plants was vacuumed to remove seeds in order to monitor regrowth of individual plants. Plants within treatments receiving a sequential application were left undisturbed. The sequential application was applied 9 August, 2001 as described above. All above-ground plant portions and seeds were removed 6 weeks after the sequential treatment as previously described and regrowth of all plants was measured 88 DAIT.

A 1-$m^2$ frame and camera support was constructed from 2.5 cm PVC pipe. This support was used to position the camera 1 m above the soil surface and in the center of the frame. An in-ground grid system was constructed to ensure that the digital images were taken consistently from the same position.

Images were collected using a Nikon 990 digital camera with a wide-angle lens attachment. With the camera set on the normal mode, the resulting images contained 1.71 million pixels per $m^2$. Each plant in every plot within the two replications was photographed before herbicide treatments were applied and again, 19, 30 DAIT and approximately monthly thereafter to evaluate Virginia buttonweed response to herbicide treatments and removal of above ground tissue. Digital images were subjected to ERDAS IMAGINE 8.5 ®[3] and SIGMA SCAN PRO 5.0®[4] for analysis. These software

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[3] ERDAS IMAGINE is a trade mark of ERDAS, Inc., 2801 Buford Highway, N. E. Atlanta, Georgia, 30329-2137.

[4] SIGMA SCAN is a trademark of SPSS Science Software, 233 S. Wacker Drive, 11th floor Chicago, IL 60606-6307.

programs were used to determine the portion of the square meter occupied by the Virginia buttonweed plants over time.

Each ERDAS IMAGINE 8.5® image was renamed from the numerical order in which it was taken to an alphanumeric system with respect to plot and plant number i.e.101-a. Each image was manually cropped to include only the area inside of the 1-$m^2$ frame. After renaming and cropping, a signature file was created for each day of data collection. The signature files enabled the user to assign a name to various colors detected and differentiated by the software. The three main objects detected were the shadows from the plants and frame, green vegetation, and bare soil. Once the signature file was successfully created, the images were processed using the batch option. The numerical pixel data was then transferred to an Excel spreadsheet where the percent of 1 $m^2$ that contained Virginia buttonweed was determined by dividing the Virginia buttonweed pixels by the total number of pixels.

SIGMA PRO 5.0 was also used to analyze the previously described images. A renaming and cropping program was written to automatically rename and crop each image. Instead of creating a signature file as in the ERDAS IMAGINE 8.5 program, the hue and saturation levels were adjustable to detect variation within ranges of desired colors. The hue and saturation ranges were 57 to 107 and 20 to 100, respectively. Another program was written to calculate the percent of the 1 $m^2$ occupied by Virginia buttonweed and these data were automatically transferred to an Excel worksheet.

**Data analysis.** Data were analyzed using mixed models analysis of variance techniques as implemented in the SAS® procedure mixed (Littell et al. 1996). Mixed

models analysis has many advantages over the traditional generalized linear models (GLM) technique. The mixed procedure uses an iterative restricted maximum likelihood approach to estimate model solutions. It is superior because it offers a way to handle violations of implicit assumptions. One assumption that is commonly violated in herbicide trials is that all treatments have homogeneous variances. This is clearly not the case because of the negative association between efficacy and error. Mixed models procedures are able to handle these situations because treatments can be grouped based on common error variances. Our approach was to first analyze a given data set under the assumption of equal variances for all treatments and recording the magnitude of the model fit statistics. We then grouped treatments based on the size of the within treatment variance and repeated the analysis with these groupings using the 'REPEATED / GROUP=VARGRP' statement within SAS® PROC MIXED, where VARGRP represents a number from 1 to the total number of treatments. If the second analysis resulted in better-fit statistics, this model was then chosen for the final analysis. The result of this type of refined analysis is that (a) only probability values are printed without either Type I or Type III sums of squares, and (b) least squares treatment means are reported with different standard errors. Least square means were used for regression analysis of the relationship between the two evaluation methods.

## RESULTS AND DISCUSSION

Both ERDAS IMAGINE 8.5 and SIGMA PRO 5.0 were able to differentiate Virginia buttonweed from its surroundings and provided numerical plant coverage data from pixel

association. Although efforts were made to produce uniform plants for evaluation, both analysis systems detected varying plant coverage before herbicides were applied. For example, percent Virginia buttonweed cover within each 1 $m^2$ initially ranged from 12.2 to 9.3%.

There was a significant difference in plant size for each date photographs were taken (Figure V. 1). There was no method by treatment interaction and thus no difference between the two software systems to rank treatments relative to percent Virginia buttonweed ground cover. After herbicide application (s), varying plant coverage was correlated to effectiveness of herbicides. Herbicides reduced plant coverage compared to the non-treated on evaluation dates 2, 3, and 4.

Data for the last set of photographs (date 5) is percent Virginia buttonweed regrowth after all above ground plant tissue had been removed. This corresponds to 85 days after removal from treatments receiving only one herbicide application and 55 days after those receiving two applications. Examples of regrowth showed non-treated plants had regrown by date 5 to cover 5.9% of the 1 $m^2$, which was equivalent to 50% of the original plant size. Intermediate efficacy was produced by two applications of metsulfuron and these plants had regrown to 3.3% (33% of original plant size) by this date. High efficacious was produced by one application of triclopyr + clopyralid + diflufenzopyr. Average regrowth for this treatment was 2.2%. However, this average is misleading since only one plant of the twelve produced regrowth. Both software programs were unable to achieve a 0% Virginia buttonweed infestation for any plot.

With the exception of date 3, there were significant differences in data generated by ERDAS IMAGINE 8.5 and SIGMA SCAN PRO 5.0. Regression of SIGMA SCAN PRO 5.0 against ERDAS IMAGINE 8.5 produced a negative intercept estimate, which indicated a consistently higher Virginia buttonweed content for ERDAS IMAGINE 8.5 than SIGMA SCAN PRO 5.0. The magnitude of difference varied by date and ranged from 0.15 to 3.46% (Table V. 1).

Although both systems provided precise objective quantifiable data, more time was required to obtain these results compared to visual estimates. The SIGMA SCAN PRO 5.0 program was more user friendly than ERDAS IMAGINE 8.5 and thus required less time to complete the analysis. SIGMA SCAN PRO 5.0 allowed for the creation of macros that reduced the laborious requirements. These programs can detect and quantify plant size or ground cover when there is a vivid contrast between the object of interest and its surroundings. When the Virginia buttonweed plants were in stressed environments from either herbicide application or natural senescence, the images were more challenging to analyze. Stressed plants turn purple and thus the analysis program had to identify two colors that represented a single object.

ERDAS IMAGINE 8.5 is a powerful program and has been utilized for many applications. Most commonly when ERDAS IMAGINE 8.5 is utilized, images are taken from remote areas such as satellites and airplanes. The average pixel size from these remote images is 1 $m^2$ where as our pictures contained 1.71 million pixels per $m^2$. This increase in image definition may not be a favorable characteristic for analysis for the

ERDAS IMAGINE 8.5 analysis system due to the challenge of selecting such small individual pixels for association assignment.

Though we were able to successfully utilize both of these image analysis programs to quantify Virginia buttonweed plant size in each plot, it was not as efficient as visual rating. Though the SIGMA SCAN PRO 5.0 was less labor intensive, it still required four times longer to process images than visual observations. Additional refinements may require further calibration such as that suggested by Richardson et al. (2001). Since this type of data analysis is more time consuming than visual observations, more work is needed to improve efficiency. However, if quantitative non-destruction data are experimental design essentials, this technique could be very effective.

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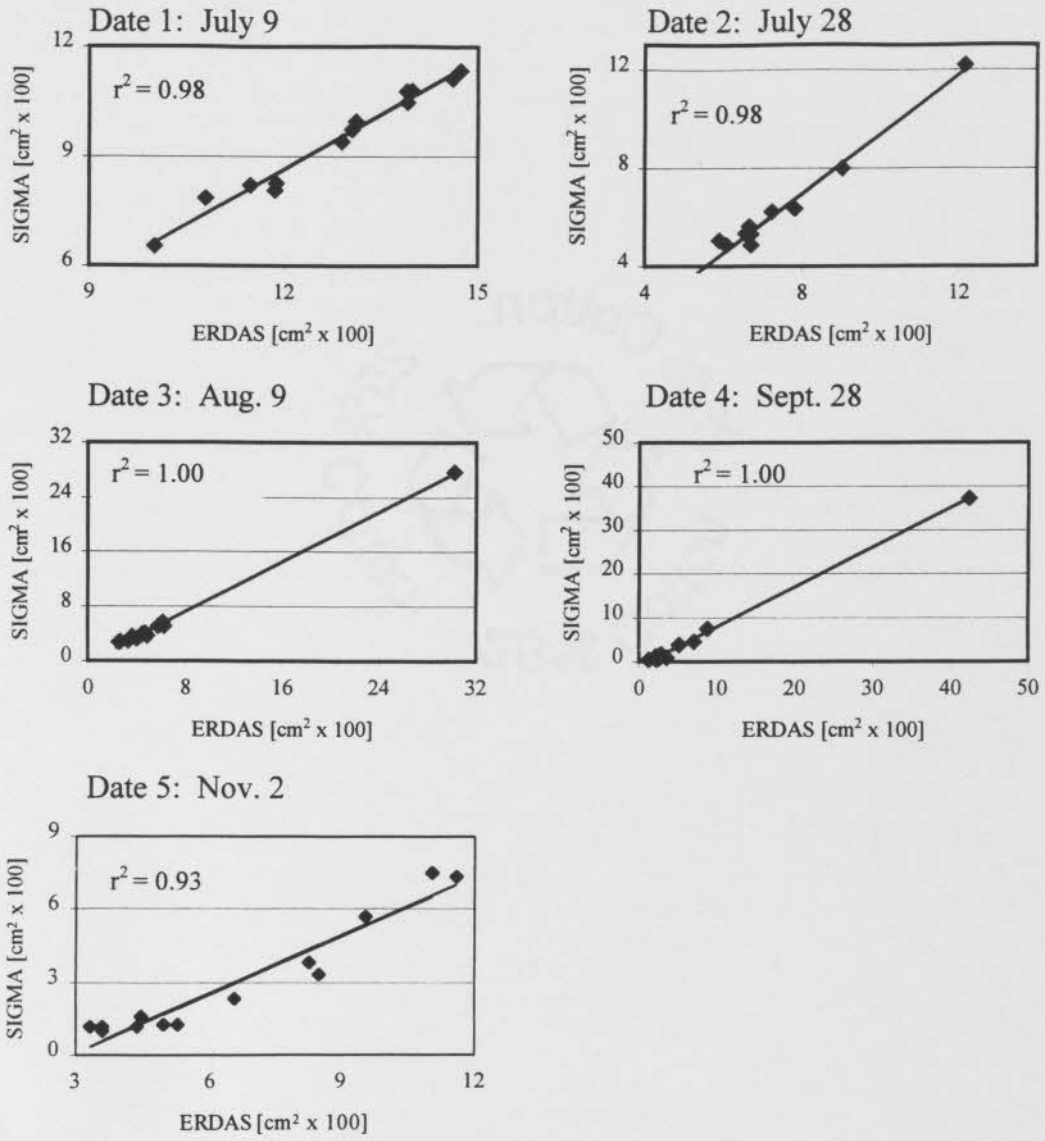


Fig. V. 1. Regression of SIGMA on ERDAS for percent Virginia buttonweed cover in 1 $m^2$ at five dates during 2001. Regression estimates and associated statistics are listed in Table V. 1.

**Table V. 1.** Intercept and slope estimates and associated statistics from the regression of SIGMA on ERDAS. The model was significant at $P = 0.001$ for all dates. The $P$-value for intercepts represents $H_0$: a = 0, whereas the P-value for slopes tested $H_0$ b = 1, i.e., there is a 1:1 relationship between SIGMA and ERDAS calculations.

| | Intercept | | | Slope | | | | |
|---|---|---|---|---|---|---|---|---|
| Date[a] | Estimate | SE | P-value | Estimate | SE | P-value | Residual | $r^2$ |
| 1 | -3.46 | 0.61 | <.0001 | 1.01 | 0.05 | 0.867 | 0.24 | 0.98 |
| 2 | -2.78 | 0.39 | <.0001 | 1.21 | 0.05 | 0.002 | 0.37 | 0.98 |
| 3 | -0.15 | 0.14 | 0.3075 | 0.92 | 0.01 | <.0001 | 0.37 | 1.00 |
| 4 | -1.26 | 0.18 | <.0001 | 0.91 | 0.01 | <.0001 | 0.54 | 1.00 |
| 5 | -2.29 | 0.48 | <.0001 | 0.80 | 0.07 | 0.013 | 0.68 | 0.93 |

[a] Date 1 = 1 day prior to initiating herbicide treatments; Date 2, 3, 4, and 5 = 19 days after initial treatment (DAIT), 30 DAIT, 88 DAIT, and 115 DAIT, respectively.