Image analysis of leafy spurge (Euphorbia esula L.) cover
by Jennifer Lee Birdsall

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Range Science
Montana State University
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Abstract:
In this study we examined whether image analysis could separate leafy spurge from other plant species and objects by comparing the image analysis and ocular methods of estimating cover. Objectives were to test image analysis and ocular leafy spurge cover estimates for repeatability and reliability, determine error levels and sample sizes based on the image analysis and ocular cover estimates, and relate the image analysis and ocular cover estimates to leafy spurge biomass. In 1993 and 1994, 1440 ocular leafy spurge cover estimates and 1440 color prints were taken at three sites. Two observers ocularly determined cover from each print. A cover estimate was also determined for each print using image analysis. The precision of the methods was tested by examining repeatability and reliability. Error levels and sample sizes were also calculated for the ocular and image analysis methods. Image analysis had the same repeatability as the ocular method ($P = 0.57$) and the image analysis estimates were reliable, differing by less than 7% from the mean ocular cover values ($P = 0.01$). Image analysis underestimated cover at high levels compared with the ocular estimates ($P = 0.00$). The average error levels of the image analysis and ocular methods did not differ ($P = 0.36$). The average number of quadrats required for image analysis to estimate cover with a 10% error was 50% lower than that of the ocular method ($P = 0.00$). Neither the image analysis nor the ocular cover estimates were reliable predictors of leafy spurge biomass. Image analysis was recommended as a measurement tool because quantification is rapid, the equipment is inexpensive, and the color prints would provide a permanent photo record of the study.

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A thesis submitted in partial fulfillment
of the requirements for the degree

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Master of Science

in

Range Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 1995

N378
B5327

APPROVAL

of a thesis submitted by

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date 28 November 1995

ACKNOWLEDGEMENTS

Sincere thanks to P. Chuck Quimby, Jr. for his mentoring, Bok Sowell for his enthusiasm, and Bret Olson, Robert Nowierski, and Tony Svejcar. Thanks to Tom Kalaris for his support and suggestions, Greg Kalaris and Norma Irish for their perspectives, and to the rest of my family. Thanks also to Norm Rees, Kim Mann, Lance Bigelow, and Kara Paintner.

TABLE OF CONTENTS

Page

LITERATURE REVIEW 1

Introduction 1
Vegetation Measurements Used in Biocontrol Studies 1
Ocular Estimates of Plant Canopy Cover 3
Image Analysis of Plant Canopy Cover 8
Reflectance and Image Analysis of Leafy Spurge 19

IMAGE ANALYSIS OF LEAFY SPURGE COVER 21

Introduction 21
Materials and Methods 23
Study Areas 23
Plot Design 23
Ocular and Image Analysis Cover Comparisons 24
Ocular Cover Estimates 24
Image Analysis Cover Estimates 25
Precision 27
Error Level and Sample Size 29
Ocular Cover, Image Analysis Cover, and Biomass Comparisons 29
Biomass and Cover Estimates 29
Biomass Models 30
Results and Discussion 30
Precision 30
Repeatability 30
Reliability 32
Error Level and Sample Size 41
Biomass Models 43
Conclusions 47

LITERATURE CITED 51

APPENDIX 56

LIST OF TABLES

Table Page

1. Number of one m^2 quadrats needed to achieve error levels of 5, 10, and 15% at the 95% significance level for different ranges of crop cover measured by image analysis 17

2. Comparison of the mean standard deviations of the ten image analysis leafy spurge cover estimates and ten ocular leafy spurge cover estimates made by each of four observers from ten color prints 31

3. Correlation coefficients between the image analysis and ocular leafy spurge cover estimates made by three observers with both years and all sites combined 33

4. Mean cover values and nonparametric groupings of the image analysis and ocular leafy spurge cover estimates made by the three observers with both years and all sites combined 34

5. Correlation coefficients between the 1993 and 1994 image analysis and ocular leafy spurge cover estimates made by the three observers at the Fergus County, Custer County, and Valley County sites 35

6. Mean cover values and nonparametric groupings from the 1993 and 1994 image analysis and ocular cover estimates made by three observers at the three sites 37

7. Mean cover values and nonparametric groupings of image analysis and ocular leafy spurge cover estimates made by three observers at low, medium, and high cover levels 40

8. Summary of the error levels for the 1993 and 1994 image analysis and ocular leafy spurge cover estimates made by the three observers at the 18 plots..... 41

LIST OF TABLES

Table Page

9. Summary of the sample sizes needed to estimate leafy spurge cover within a 10% error at a 95% confidence level based on the 1993 and 1994 image analysis and ocular leafy spurge cover estimates made by the three observers at the 18 plots 42

10. Correlation coefficients between the 1994 image analysis estimates of leafy spurge cover, the ocular leafy spurge cover estimates made by the three observers, and the leafy spurge biomass estimates from the northwest transects at all sites combined 44

11. Correlation coefficients between the 1994 image analysis estimates of leafy spurge cover, the ocular estimates of leafy spurge cover made by the three observers, and the leafy spurge biomass estimates from the northwest transects at the Fergus County, Custer County, and Valley County sites 46

A-1. List of observed plant species at the Fergus County, Montana site 57

A-2. List of observed plant species at the Valley County, Montana site 58

A-3. List of observed plant species at the Custer County, Idaho site 59

LIST OF FIGURES

Figure Page

1. Daubenmire frame and mounted 35 mm camera used to generate color prints 24

2. The image analysis system consisting of a video camera, an image display screen, a computer containing the electronics for receiving the image, a terminal, and software 26

3. Binary image where pixels with grey values that correspond to leafy spurge appear white and remaining pixels appear black with original print in lower right corners for comparison 27

4. Image analysis estimates of leafy spurge cover in relation to ocular estimates of leafy spurge cover made by Observer 1 from color prints, by Observer 2 from color prints, and by Observer 3 from field observations 32

5. Biomass of leafy spurge in relation to estimates of leafy spurge cover made using image analysis, and ocular estimates made by Observer 1 from color prints, by Observer 2 from color prints, and by Observer 3 from field observations 45

ABSTRACT

In this study we examined whether image analysis could separate leafy spurge from other plant species and objects by comparing the image analysis and ocular methods of estimating cover. Objectives were to test image analysis and ocular leafy spurge cover estimates for repeatability and reliability, determine error levels and sample sizes based on the image analysis and ocular cover estimates, and relate the image analysis and ocular cover estimates to leafy spurge biomass. In 1993 and 1994, 1440 ocular leafy spurge cover estimates and 1440 color prints were taken at three sites. Two observers ocularly determined cover from each print. A cover estimate was also determined for each print using image analysis. The precision of the methods was tested by examining repeatability and reliability. Error levels and sample sizes were also calculated for the ocular and image analysis methods. Image analysis had the same repeatability as the ocular method ($P = 0.57$) and the image analysis estimates were reliable, differing by less than 7% from the mean ocular cover values ($P = 0.01$). Image analysis underestimated cover at high levels compared with the ocular estimates ($P = 0.00$). The average error levels of the image analysis and ocular methods did not differ ($P = 0.36$). The average number of quadrats required for image analysis to estimate cover with a 10% error was 50% lower than that of the ocular method ($P = 0.00$). Neither the image analysis nor the ocular cover estimates were reliable predictors of leafy spurge biomass. Image analysis was recommended as a measurement tool because quantification is rapid, the equipment is inexpensive, and the color prints would provide a permanent photo record of the study.

LITERATURE REVIEW

Introduction

In biological control of weed programs, researchers or land managers monitor the establishment and effects of introduced weed attacking agents. The effects of these agents on vegetation have been assessed with several measures, each with inherent strengths and weaknesses. Currently, researchers often assess these effects by ocularly estimating plant canopy cover. While ocular plant canopy cover is useful to describe trends in vegetation (Brown 1954), ocular cover estimates can be highly biased (Bonham 1989, Schultz et al. 1961). Image analysis may be a method to determine plant canopy cover with less bias (Lindsey and Bassuk 1992, Tagliavini et al. 1993).

Vegetation Measurements Used in Biocontrol Studies

In early biological control programs researchers often used photographic techniques to measure effects on vegetation (Rees 1978, Vogt et al. 1992). Photographs are simple, rapid, inexpensive, and objective but difficult to quantify statistically (Bonham 1989, Pitt and Schwab 1990). Problems with clarity and scale can reduce the value of photographs as a vegetation measure (Owens et al. 1985).

Density is an easily understood quantitative measure that researchers have used in biological control programs (Brosten and Sands 1986, Coombs et al. 1991). Although density remains more constant from year to year than cover or biomass (Bonham 1989), counting can be slow and distinguishing individual plants can be difficult (Harper 1977, Room 1985). Moreover, density does not provide information on a species' relative influence in a community (Kok and Pienkowski 1985).

Determining age-class distributions is an extension of density which has been used in biological control programs (McEvoy 1985, Pemberton and Turner 1990, Reynolds 1992). With this method, plant counts are separated into stages (i.e. seedling, rosette, flowering). As biological control agents can affect plant architecture and hence reproductive potential without affecting overall density, this technique may be more sensitive than density alone (Room 1985).

Plant biomass has been used to evaluate the effects of biological control agents (Harris 1991, Johnson et al. 1992, McEvoy 1985). While biomass is highly correlated with competitive ability (Gaudet and Keddy 1988, Harris 1989), the measures are tedious and expensive and biomass may vary seasonally and yearly (Bonham 1989).

Plant cover measures have frequently been used to evaluate the effects of biological control agents on vegetation (Frank 1991, Quimby and Antognini 1992, Rees 1991, Reynolds 1992). Plant cover measures are obtained quickly and provide quantitative data on a species' relative influence in a community (Daubenmire 1959). Drawbacks include yearly and seasonal fluctuations in cover and the high bias associated with visual estimates (Bonham 1989).

Ocular Estimates of Plant Canopy Cover

Ocularly or visually estimating plant canopy cover is a technique commonly used in biological control programs to assess effects of biological control agents on vegetation. Researchers use this technique because ocular plant canopy cover measures are correlated with phytomass and are easier to obtain than weight or density (West 1983). The quadrat area method of estimating aerial plant canopy cover is currently the most frequently used ocular cover method (Harris 1991, Johnson 1992, Rees 1991).

Brown (1954) described cover as the area covered by the vertical projection of aboveground plant parts expressed in relation to the total ground surface, and indicated that cover is the best criterion for ascertaining succession, trends following certain practices or treatments, or other ecological changes. Daubenmire (1959) defined canopy cover as the percentage of ground covered when a polygon drawn about the extremities of the undisturbed canopy of each plant is projected upon the ground and all such projections are summed. Daubenmire (1959) indicated that the canopy cover method, in addition to being efficient, best meets the objectives of being able to evaluate a taxon's relative influence in the community, compare plants of widely different life forms on the same basis, and sample with sufficient accuracy so that successional trends can be evaluated. Schlatterer (1983) related that canopy cover estimates are useful in ecological research and ecosystem classification.

Cover can be estimated for the plant canopy or the plant base. Aerial or canopy cover is the maximum leaf spread (Brown 1954) and is generally used to measure

vegetative cover when the stem is smaller than the aerial spread or crown (Pieper 1978). Canopy cover estimates are common in biological control of weeds studies because many weeds are broadleaved, herbaceous perennials. Basal cover or the cover at ground level is considered more reliable for tussock or bunchgrasses and is less subject to changes resulting from animal use and short-term climatic variation (Brown 1954, West 1983). Canopy cover is correlated with biomass and forage availability, while basal cover is associated with density (West 1983). Variations exist in the method of estimating canopy cover (Hutchings and Pace 1963). Some estimators consider aerial cover as the gross crown spread regardless of any openings (Daubenmire 1959, Kinsinger et al. 1960) whereas others adjust the measurement to account for foliar openings (Hutchings and Pace 1963).

To monitor the effects of biological control agents on weeds, canopy cover is most often estimated using the quadrat area method rather than transects or points (Brown 1954). The quadrat area method utilizes a small quadrat or frame that is viewed from above. The proportion of ground covered by vegetation, rocks, litter, or any other material is estimated (Hutchings and Pase 1963). The size, shape, and number of quadrats depend on factors such as the degree of accuracy desired, vegetation type, funding, and available labor ('t Mannetje 1978).

Quadrats of various sizes have been used to measure cover depending on the size and diversity of the plant species present in the study area. In general, plants with high biomass can be measured with fewer larger plots than vegetation with lower biomass (Brown 1954). However, in diverse plant communities, the use of smaller but more

numerous quadrats may detect more species (Daubenmire 1959). Daubenmire (1959) recommended a 0.1 m^2 quadrat for vegetation up to one meter in height, rather than the larger quadrats previously used. He claimed that at quadrat sizes larger than 0.1 m^2 the eyes are required to move more and memory becomes involved, increasing the error. At smaller quadrat sizes the numbers required for an adequate sample increase rapidly. While research has been published on the effects of quadrat size on frequency and biomass, few experiments have tested effects of quadrat size on cover (Mitchell et al. 1988).

Quadrat shapes include square, rectangular, and circular. Many sampling errors arise from edge effects due to problems with defining boundaries and disturbing vegetation ('t Mannetje 1978). To reduce edge effects, the quadrat perimeter should be as small as possible in relation to the area of the sampling unit. The perimeter to area ratio is smallest for a circle and some researchers have recommended circular quadrats (Van Dyne et al. 1963). However, circular quadrats can be difficult to place anywhere other than in low vegetation, such as short grasslands (Brown 1954, t' Mannetje 1978). In taller vegetation, it is more practical to use an open-ended square or rectangle. Researchers have long recognized that rectangular quadrats represent the variation in a stand better than squares (Oosting 1956) and favor rectangular quadrats on statistical grounds (t' Mannetje 1978). Daubenmire (1959) recommended rectangular quadrats with inside dimensions of 20 by 50 cm.

The number of quadrats to use for an accurate estimate varies and depends on vegetation type. Daubenmire (1959) recommended using at least 20, but preferably 40, 0.1 m^2 quadrats to sample a large percentage of the taxon with sufficient accuracy.

Greig-Smith (1964) presented a method to determine the optimal number of sampling units. The mean of the variable being estimated is plotted against the number of quadrats. The flux of the mean values will decline as number of quadrats increase. The final number of quadrats used is generally a compromise between accuracy, practicality, and cost (t' Mannetje 1978).

With the quadrat area method, a researcher can estimate the proportion of the plot covered by each species directly as a percentage or can use cover classes where each observation is recorded as equal to the midpoint of the class in which it falls (Mitchell et al. 1988). Daubenmire (1959) recommended using six cover classes (0 to 5, 5 to 25, 25 to 50, 50 to 75, 75 to 95, and 95 to 100%), believing that with cover classes defined so broadly there should be little chance for consistent human error. However, Mitchell et al. (1988) found that when macroplots were sampled, the Daubenmire cover class observations overestimated cover when the canopy was relatively open and underestimated cover when the canopy was more dense. The investigators determined that these errors were not due solely to plot size. The ranges of the cover classes and the sample size also affected the results. Mitchell et al. (1988) found that the sensitivity of the cover class method to detect changes in vegetative cover depended on cover class widths. The greatest potential for error existed when the cover classes had unequal widths.

The accuracy of the canopy cover estimates using the quadrat area method is affected by the method used to determine cover and by the size, shape, and number of quadrats studied. However, another source of error is introduced when canopy cover is estimated ocularly. Schultz et al. (1961) used an artificial population board to compare

ocular, line intercept, line point, and loop methods of estimating cover. Ocular estimation had the highest bias of the methods tested, overestimating cover by one-third more than the true value. Despite the higher bias, ocular estimates of percent cover are the most often used quadrat method (Bonham 1989).

Researchers have long recognized that the accuracy and precision of ocular estimates depend on the subjectivity of the individual estimator (Fisser and Van Dyne 1966, Smith 1944). An accurate technique would estimate a true population parameter, while a precise technique gives repeatable results (Bonham 1989). Significant bias can exist between estimators, days, vegetation types, and between the same estimator during a single sampling period (Smith 1944, Hutchings and Pase 1963). While there are individuals who are able to estimate cover with a high degree of accuracy, most estimators lack accuracy and precision ('t Mannetje 1978). Schultz et al. (1961) used inexperienced high-school students and experienced estimators on an artificial population board to demonstrate that the amount of bias does not seem to decrease with experience. This constant bias may result from the inability of the estimators to compare their ocular estimates against accurate measures ('t Mannetje 1978, Schultz et al. 1961). Other researchers have reported that bias decreases when double sampling is used, where ocular estimates are coupled with checks against actual yields (Campbell and Arnold 1973, Pechanec and Pickford 1937). However, double sampling is time consuming and costly and does not eliminate bias.

Clearly, the ocular method of estimating plant canopy cover can be inaccurate and imprecise. 't Mannetje (1978) described cover as entirely subjective and lacking in

repeatability. Kinsinger et al. (1960) stated that no accurate method of measuring plant cover has been devised. As a result, there exists a need for a rapid, accurate, and easy to use method of measuring plant cover. Image analysis may be able to address this need.

Image Analysis of Plant Canopy Cover

Image analysis is an electronic technique in which a video image is digitized or numerically converted to permit computer analysis. In black and white image analysis, specific picture elements of the video image (pixels) are sequentially isolated using a grid scanning pattern. Each pixel is assigned a data quantization level, termed a grey level. An operator specifies a subset of grey levels which correspond to the objects targeted for classification and the computer quantifies the proportional area occupied by the pixels within the subset (Gerten and Wiese 1987). Image analysis is based on the assumption that the target objects being classified have sufficiently different reflectance coefficients than the nontarget objects within the video image, and that the digitizing hardware and software can make the distinction (Meyer et al. 1988, Shelton et al. 1988). The fundamental purpose of image analysis is to digitize, store, and process an image to extract information about a specific response (Stutte 1990).

The first step in image analysis is to generate a photographic or electronic image (Grotta and Grotta1992). Most researchers generate the video images using film based cameras because these cameras are inexpensive and have high overall image quality. All photographs and most videos are composed of analog data consisting of continuously variable light, color, and contrast signals. Since computers are digital devices, the analog

images must be converted into digital data consisting of numerical signals (zeros and ones). The two types of devices used to convert analog to digital images are scanners and frame grabbers. A digital image typically consists of 512 by 512 pixels, each assigned a grey level between 0 and 255 (256 levels total). The digital image is then fed into a computer. If necessary, the image can be enhanced at this point. Numerous enhancement algorithms exist which range from simple contrast optimizing, smoothing, or sharpening and edge detection to more complicated, such as arithmetic and logical operators (Lennard 1990).

The second step in image analysis is to segment the image. In this step the objects of interest are separated from the background. The simplest way to segment an image is to define a threshold of grey values which best correspond to the objects of interest. Pixels with grey levels within the threshold are classified into one group while pixels outside the threshold are classified into another (Tillett 1991). When a threshold is defined, a new image is created on the monitor so that comparisons can immediately be made with the original image (Lindsey and Bassuk 1992). The new image is binary with all the pixels in the threshold appearing white and all the pixels outside the threshold appearing black. The white pixels are the objects which have been selected for further processing. As the actual greyness levels of the objects are not needed, subsequent processing is done on the binary image (Tucker et al. 1992).

The final step in image analysis is to quantify the objects of interest. An operator can count objects or measure variables such as area, length, slope, angle, intensity, perimeter, or *x-y* coordinates (Rundel 1992). In the future, the fields of artificial

intelligence and expert systems may enable this step to include image understanding and interpretation (Tillett 1991).

Recent advances in computer hardware and software are eliminating the barriers to use of image analysis. Image analysis previously required a mainframe or at least a minicomputer, a resident programmer, and expensive and cumbersome software packages (Lennard 1990, Grotta and Grotta 1992). Inexpensive image analysis software is now available for personal computers and does not require a resident programmer. Experts predict that image analysis programs will continue to become easier to use and less expensive (Grotta and Grotta 1992). The equipment needed for image analysis currently includes a frame grabber board or scanner, a computer, and software.

Frame grabbers and scanners convert analog to digital images. Frame grabbers fit inside the computer and range in price from $200 to $2,500 (Grotta and Grotta 1992, Krumenaker 1994). Scanners vary in size, shape, and sophistication and range in price from $150 to $15,000 (Grotta and Grotta 1992). Recently, filmless electronic cameras have been developed which directly transmit digital signals. The image is stored on a computer disk rather than on film. Since these cameras do not require film developing with silver-based emulsions, filmless cameras are environmentally friendly. These cameras currently range in price from $200 to over $20,000 (Grotta and Grotta 1992, Krumenaker 1994). Eventually filmless cameras may supersede film-based cameras and a scanner or frame grabber would no longer be needed.

The computer must be at least a 386-level Macintosh or IBM-PC compatible system with eight megabytes of random access memory, a 24 or 32 bit graphics board,

a monitor, and ten megabytes of free disk space (Grotta and Grotta 1992, Anonymous 1994). Image analysis software packages range in price from $295 to tens of thousands of dollars. Most packages sell for several thousand dollars (Krumenaker 1994, Meisel and Recchia 1988).

Use of image analysis in agriculture and biology has increased since the 1970s with advances in image analysis hardware and software. Sader and Winne (1991) found that 31 of 44 surveyed forestry departments at United States universities operated at least one image analysis program in 1988. Image analysis has been used in plant science to quantify plant disease symptoms, measure spray droplet sizes on a crop canopy, determine plant organ areas in physiological and morphological studies, measure root lengths and widths, grade produce, and classify and quantify vegetation and soil (Yanuka and Elrick 1985, Stutte 1990, Tagliavini et al. 1993). The following studies highlight some of the uses of image analysis in the plant sciences and detail methods used to compare image analysis measures with measures obtained conventionally.

Many researchers have compared image analysis with conventional measures by correlating or regressing image analysis measures with conventional measures. Yanuka and Elrick (1985) measured root lengths and widths with image analysis and with conventional methods and linearly correlated these measures. The correlation between the conventional root length measures and the image analysis measures had an r-value of 0.99. The root width correlation had r-values of 0.90 and 0.95 depending on the size of the pixels selected in the image analysis program. Tagliavini et al. (1993) measured peach [*Prunus persica* (L.) Batsch.] root surface areas and diameters conventionally and with

image analysis. The researchers used correlation to compare the conventional measures with the surface area measures determined by image analysis. The measures were highly correlated with an r-value of 0.95. Image analysis root diameters were in close accord with calculated diameters and the image analysis method had a smaller coefficient of variation.

The precision of image analysis measures has been examined in several studies. McMillan and Schwartz (1993) used image analysis to quantify bean (*Phaseolus vulgaris* L.) leaf damage due to a pathogen. The researchers demonstrated the reproducibility of the image analysis measures by repeating the measures many times for each leaf but did not perform any statistical analyses on the repeated measures. In another study, Nutter et al. (1993) quantified the precision of image analysis and visual estimates of percent disease severity of dollarspot (*Sclerotinia homoeocarpa* F. T. Bennett) on bentgrass (*Agrostis palustris* Huds.). Four raters quantified percent disease severity visually from color slides of one m^2 quadrats by dividing the diseased area of the quadrat by the total quadrat area. Another rater quantified percent disease severity from the slides using image analysis. All five raters quantified each slide twice on different days. Precision was defined as the relative measure of the repeatability and reliability of the estimates. The researchers determined repeatability among raters by regressing the first and second estimates of each observer. The linear regression R^2-value on the first and second image analysis estimates was 0.63. The R^2-values of the first and second visual estimates ranged from 0.80 to 0.93. The reliability of the visual measures was determined by examining the between-rater agreement of the measures using linear regressions. The R^2-values varied

from 0.77 to 0.89, although numerous disagreements existed. For example, rater one consistently overestimated percent disease compared with the other raters. Slopes, *y*-intercepts, and coefficients of variation were used to further compare repeatability among raters and reliability between raters..

Some studies have discussed the time required to perform image analysis. McMillan and Schwartz (1993) initially spent a high amount of time acquiring images of bean leaves and performing image analysis on the images. However, after developing a routine, the researchers could obtain and analyze an image in 20 to 30 seconds. Thomas et al. (1988) found that it took 52 seconds on average to visually inspect a slide and 104 seconds for image analysis. The time required to visually inspect a slide increased as percent cover increased but remained relatively constant for image analysis. Thomas et al. (1988) also noted that visually inspected data are generally recorded on paper while image analysis data are recorded on computer. Additional processing time would be needed to enter the visual data onto a computer.

The ability of image analysis to separate and identify objects has been examined. Ruark and Bockheim (1988) tried to use image analysis to separate aspen (*Populus tremuloides* Michx.) tree roots from other objects. The researchers first sprayed the area with Rhodamine WT dye which is preferentially absorbed by roots and obtained area images using color infrared film. Image analysis could separate roots from soil and rocks 98 to 100% of the time. Occasionally, protruding angles of stones reflected light similarly to the roots. The stones were visually evident on the monitor and the researchers were able to edit the stones from the selected threshold. However, image analysis was unable

to separate aspen roots from roots of other species. Guyer et al. (1986) used image analysis to determine if differences in numbers and shapes of leaves could be used to identify individual species. By measuring the ratio of the square perimeter to the area of a leaf, the researchers could use image analysis to identify corn (*Zea mays* L.), soybean (*Glycine max* L.), tomato (*Lycopersicon esculentum* Mill.), Jimsonweed (*Datura stramonium* L.), velvetleaf (*Abutilon threophrasti* Medic.), and lambsquarters (*Chenopodium album* L.). The grass species, giant foxtail (*Setaria faberi* Herrm.) and Johnsongrass [*Sorghum halepense* (L.) Pers.], were difficult to differentiate. Image analysis could separate these grasses using additional parameters. Guyer et al. (1986) concluded that the potential exists for image analysis to separate individual plant species.

Some researchers have compared film types used in image analysis. Gerten and Wiese (1987) used image analysis to measure lodging in winter wheat (*Triticum aestivum* L. em. Tell.) from true color and color infrared photos. The true color and color infrared photos were equally straightforward to interpret and both resulted in reasonably accurate image analysis estimates when compared with manually prepared photo interpretations. Image analysis of the photos underestimated lodging by only 4.35 to 7.5%. Gerten and Wiese (1987) speculated that a camera equipped with shading compensators may have improved the image analysis measures. Meyer and Davison (1987) obtained black and white and color infrared photos of individual leaves and of quadrats in the field. Image analysis of the black and white photos could not always distinguish between vegetation and soil. Image analysis of the color infrared photos separated vegetation and soil more often but infrared film is more expensive and has stricter handling requirements.

Meyer et al. (1988) and Shelton et al. (1988) compared the ability of black and white and color image analysis programs to estimate percent soybean residue cover. Color slides were shot of 0.76 by 1.20 m quadrats using a tripod-mounted camera one meter above the soil surface. The researchers shaded the quadrats from direct sunlight and used a flash for uniform lighting conditions. The image analysis cover estimates were compared using linear regression analysis with cover estimates obtained using the conventional photographic grid method. The R^2-values were 0.92 for color image analysis and 0.75 for black and white image analysis. Color image analysis separated residue from background objects more reliably than black and white image analysis.

Image analysis has been used to determine canopy cover in several other studies. Thomas et al. (1988) used image analysis of color slides to measure percent canopy cover of rapeseed (*Brassica napus* L.). To determine accuracy, the researchers compared image analysis and visual estimates of the canopy cover with cover measures obtained using a leaf area meter. The image analysis estimates were in close agreement with the leaf area meter measures. The visual estimates were higher than the leaf area meter measures but had a lower coefficient of variation than the image analysis measures. Thomas et al. (1988) determined that image analysis estimated average canopy cover more accurately than the visual method but that image analysis was less accurate at estimating canopy cover in individual photos. Meyer and Davison (1987) used image analysis to determine leaf area of soybean, sunflower (*Helianthus annuus* L.), and velvetleaf and to measure canopy closure from overhead photos. Image analysis of the leaf areas was compared with leaf area measures from the electrical optical method using linear regression analysis.

The R^2-values were 0.995 for soybean and 0.993 for sunflower and velvetleaf. While image analysis showed promise for measuring leaf area in the field, uneven lighting conditions produced shadows which were difficult to distinguish from the leaves. Plant canopies with more uniform lighting conditions were easier to measure.

Stone et al. (1988) compared the precision of image analysis and the photographic grid method of measuring crop cover. Seven observers estimated cover on 15 false color infrared photos three times using each of the two methods. A variance component model was used to estimate the reproducibility of the two methods. Stone et al. (1988) determined that the precision of the image analysis and grid methods at determining cover were the same and the methods had similar total variance.

In one study the percent cover data was transformed to logits to give normally distributed residuals and equal variances. Stone et al. (1988) used image analysis to measure percent ground cover of lettuce (*Lactuca sativa* L.), carrots (*Daucus carota* L.), and onions (*Allium cepa* L.) and compared these measures with photographic grid measures of cover. The researchers converted the percent data to logits then used functional regression analysis to compare the measures from the two methods. Ordinary linear regression was not appropriate as it requires the independent variable, which in this study was the grid measures, to be known without error. In functional regression, the model is still assumed to be $y=a+bx$ but both variables are subject to error and the ratio of their variances is used to estimate the coefficients a and b. Stone et al. (1988) determined that the accuracy of the image analysis method was dependent on the operator selecting the optimum threshold. The image analysis program had trouble discriminating between

foliage in deep shadow and soil. Use of a flash during image acquisition may have reduced this error. Individual judgement was the largest error source in the grid method.

Molloy and Moran (1991) examined error levels for the image analysis and photographic grid methods of measuring crop residue cover using a random process model. Error for the photographic grid method was 27 times as high on average as error for the image analysis method. Molloy and Moran (1991) also generated a sampling rate graph for the number of one m^2 quadrats required to obtain errors of 5, 10, and 15% at the 95% significance level for different ranges of crop cover (Table 1).

Table 1. Number of one m^2 quadrats needed to achieve error levels of 5, 10, and 15% at the 95% significance level for different ranges of crop cover measured by image analysis. Adapted from Molloy and Moran (1991).

	Error level		
Cover	5%	10%	15%
---%---			
0-20	2-7	2-5	2-4
20-40	7-19	5-7	4-6
40-60		7-12	6-7
60-80		12-19	7-10
80-100		19-28	10-14

Few studies have examined the ability of image analysis to determine plant biomass. Ernst and Kuhn (1989) used image analysis to determine leaf area of Benjamin fig (*Ficus benjamina* L.) from infrared images. The researchers then examined the relationship between leaf area estimates from image analysis and plant mass based on the principle that the number of pixels attributed to leaves should correlate with plant mass. The correlation coefficient (r) between conventionally obtained leaf area measures and image analysis leaf area estimates was 0.90. However, the image analysis leaf area measures and plant mass were not linearly correlated. An exponential model was more appropriate and produced an r-value of 0.989. Stone et al. (1984) reported a logarithmic relationship between crop cover and crop dry weight. Sydnor et al. (1975) developed a quadratic linear model relating photographic plant volume to fresh and dry weights but did not try to relate cover to biomass.

A single model to relate cover and biomass for all plant species may not exist. Bonham (1989) stated that while cover has a degree of correlation with biomass, equations must be developed for each individual species. These equations are generally of the form $y=a+b[f(x)]$ where y is the biomass estimate, a is a constant, b is the slope of the line, and $f(x)$ is some function of the measured species' cover. The function can be logarithmic or otherwise. Roebertsen et al. (1988) stated that before image analysis can be used to measure biomass, the image analysis values have to be calibrated with biomass measures for the vegetation type. After calibration, only pictures are needed to quantify changes in biomass during a growing season.

Reflectance and Image Analysis of Leafy Spurge

Images with high reflectance contrast between target objects and the background are ideal for image analysis. Studies show reflectance of individual plant species generally differ from those of other species and soil. Plants typically have low reflectance in the visible spectral region (400 to 700 nm) because of strong absorption by chlorophylls and other pigments and relatively high reflectance in the near infrared region (700 to 1300 nm) because of internal scattering due to cellular structure (Guyer et al. 1986). Knipling (1970) studied the reflectance properties of plant leaves and canopies. Leaves of each plant species tend to have a characteristic reflectance signature because of surface, thickness, internal structure, and pigment content. The reflectance of a plant canopy is similar to that of a single leaf but is generally lower because of the attenuation and non-uniformity of the solar radiation due to plant structures, leaf orientations, shadows, and background surface reflectivity. Like leaves, the canopy of each species tends to have a characteristic reflectance due to its unique structure or geometry which is determined by the size, shape, and orientation of the plants. Han and Hayes (1990) used image analysis to separate crop canopy cover from soil and found that the canopy had its highest reflectance at 550 nm whereas soil had its highest reflectance at 700 nm and higher.

Everitt et al. (1994) examined the reflectance properties of leafy spurge and the ability of image analysis to separate leafy spurge from other objects in a preliminary study. The researchers showed that leafy spurge had significantly different reflectance values in

the visible regions than associated vegetation and soil. This resulted in different digital values during image analysis of color and infrared aerial photos.

These studies indicate that image analysis may be a viable method to determine leafy spurge cover from color prints of field quadrats. A model to relate leafy spurge canopy cover as measured with image analysis with leafy spurge biomass may be able to be developed. Since stress can result in reduced total leaf area of a plant community (Knipling 1970), image analysis might provide a method to analyze the effects of biocontrol agents on leafy spurge with less bias than the ocular cover method.

IMAGE ANALYSIS OF LEAFY SPURGE COVER

Introduction

In biological control of weed programs, researchers or land managers monitor the establishment and effects of introduced weed attacking agents. The effects of these agents on vegetation have been assessed with several measures, each with inherent strengths and weaknesses. Currently researchers often assess these effects by ocularly estimating plant canopy cover. While ocular plant canopy cover can be useful to describe trends in vegetation (Brown 1954), the accuracy and precision of ocular cover estimates depend on the subjectivity of the estimator (Fisser and Van Dyne 1966, Smith 1944). Significant bias can occur between different estimators and between the same estimator during a single sampling period (Bonham 1989, Hutchings and Pase 1963). Image analysis may able to determine the effects of biological control agents on plant canopy cover with more precision than the ocular cover method.

Image analysis is an electronic technique in which a video image is digitized or numerically converted to permit computer analysis. In black and white image analysis, specific picture elements of the video image (pixels) are sequentially isolated using a grid scanning pattern. Each pixel is assigned a data quantization level, termed a grey level. An operator specifies a subset of grey levels which correspond to the objects targeted for

classification and the computer quantifies the proportional area occupied by the pixels within the subset (Gerten and Wiese 1987). Image analysis is based on the assumption that the target objects being classified have sufficiently different reflectance coefficients than the nontarget objects within the video image, and that the digitizing hardware and software can make the distinction (Meyer et al. 1988, Shelton et al. 1988). The fundamental purpose of image analysis is to digitize, store, and process an image to extract information about a specific response (Stutte 1990). Image analysis has been used in the plant sciences to quantify plant disease symptoms, measure spray droplet sizes on a crop canopy, determine plant organ areas, measure root lengths and widths, grade produce, and classify vegetation and soil (Stutte 1990, Tagliavini et al. 1993, Yanuka and Elrick 1985).

Our study examined the ability of black and white image analysis to separate leafy spurge from other plant species and background objects by comparing the image analysis method of estimating leafy spurge cover with the ocular method. Specific objectives were to: a) test image analysis cover estimates and ocular estimates made by four observers for repeatability; b) examine the similarity between image analysis cover estimates and ocular estimates made by three observers; c) determine error levels and sample sizes based on the image analysis and ocular cover estimates made by three observers; and d) relate the image analysis and ocular cover estimates made by three observers to leafy spurge biomass.

Materials and Methods

Study Areas

Study areas were in Fergus County, Montana; Valley county, Montana; and Custer County, Idaho. The Fergus County site was in the unglaciated plains. The climate is dry sub-humid with an average annual precipitation of 37.5 to 47.5 cm. Leafy spurge dominated the site with numerous grass and forb species and some shrubs (Appendix A, Table A-1). The Valley County site was in the glaciated plains with a semi-arid climate and average annual precipitation of 25 to 35 cm. Vegetation consisted of leafy spurge and grasses with some forbs and shrubs (Appendix A, Table A-2). The Custer County site was in the northern cold desert. The climate is semi-arid with an average annual precipitation of 20 to 25 cm. Vegetation was mainly leafy spurge, grasses, and sagebrush with other shrubs and forbs also present (Appendix A, Table A-3).

Plot Design

Six plots were established at each site (18 plots total). Plots were circular with 44 m diameters and minimum distances of 0.8 km between plots. Transects radiated from the center in five directions: north, south, east, west, and northwest. Ten sampling loci were marked along each transect using wooden stakes. The sampling loci began 8 m from the center point and continued every 4 m for 10 stakes. The 40 north, south, east, and west sampling loci in each plot (720 total for all plots) were used to collect image analysis and ocular leafy spurge cover data. The 10 northwest sampling loci in each plot (180 total for all plots) were used to collect biomass, image analysis cover, and ocular cover data.

Ocular and Image Analysis Cover Comparisons

Ocular Cover Estimates In the summers of 1993 and 1994, three observers ocularly estimated leafy spurge cover either in the field or from color prints taken at the 720 north, south, east, and west sampling loci (1440 total for both years). The three observers estimated non-overlapping leafy spurge cover within 0.1 m^2 Daubenmire frames (inside dimensions of 20 by 50 cm) mounted on 30 cm legs for ease of placement in tall vegetation. Leafy spurge cover was recorded to the nearest 5%.

Two of the three observers (Observers 1 and 2) estimated leafy spurge cover from color prints taken of Daubenmire frames at the sampling loci. The camera was a 35 mm single lens reflex (Olympus OM-1) equipped with a 35 to 70 mm lens and was mounted one meter above ground level (Figure 1). The photos were taken using Kodak Gold 200 film with f-stops adjusted for prevailing natural light conditions.



Figure 1. Daubenmire frame and mounted 35 mm camera used to generate color prints.

The third observer (Observer 3) ocularly estimated leafy spurge cover in the field at the 720 north, south, east, and west sampling loci. In 1993, the ocular field estimates made by Observer 3 were taken up to five weeks earlier than the color photos. In 1994, the ocular field estimates and color photos were taken concurrently.

Image Analysis Cover Estimates Leafy spurge cover was also estimated from the 1440 color prints using black and white image analysis. Image analysis was conducted on a Compudyne IBM compatible 486 personal computer equipped with a PCVISIONplus™ frame grabber board and Java™ image analysis software (Jandel Scientific 1993). The original print was transferred to the system using a COHU video camera and displayed on a separate video monitor (Figure 2).

The frame grabber board divided the digital image into 512 by 480 pixels. Each pixel corresponded to a particular (x, y) coordinate of the image and was automatically assigned a brightness or grey level in the range 0 to 255 (8 bits). Only the pixels within the Daubenmire frame were selected for image analysis. The selected pixels were segmented using a threshold of grey values. An operator (Observer 2) selected the grey value range that corresponded to leafy spurge in the image. The image was then transformed into a binary one where all pixels in the selected threshold were white and all pixels outside the threshold were black. The binary image was compared with the original print and, if the images were not similar, the operator selected a different threshold until there was close agreement (Figure 3). Leafy spurge cover was estimated by determining the percentage of white pixels.



Figure 2. The image analysis system consisting of a video camera, image display screen, computer containing the electronics for receiving the image, terminal, and software.



Figure 3. Binary image where pixels with grey values that correspond to leafy spurge appear white and remaining pixels appear black with original print in lower right corner for comparison.

Precision Precision was defined as the relative measure of the repeatability and reliability of the estimates. To compare the repeatability of the ocular and image analysis cover estimates, 10 of the 1993 prints were selected which varied in cover from 0 to 100%. Four independent observers (Observers 1 to 4) ocularly estimated leafy spurge cover 10 times for each print on different days. Leafy spurge cover was also estimated 10 times for each print using image analysis. The variable used to compare repeatability was the mean standard deviation of the ten estimates for each of the ten prints. Because the standard deviations were normally distributed (all $P > 0.11$) according to the Shapiro-Wilk test (SAS Institiute, Inc. 1987) and the variances were homogeneous ($P > 0.05$) according

to the Bartlett test (Neter and Wasserman 1974), the data were analyzed using analysis of variance (SAS Institute, Inc. 1987). The mean variances and Tukey groupings are reported for the four estimators and image analysis.

Reliability referred to the agreement between the image analysis and ocular cover estimates. To test reliability, the 1993 and 1994 image analysis and ocular cover estimates were compared using measures of correlation and Kruskal-Wallis tests (SAS Institute, Inc. 1987). Kruskal-Wallis tests were used because the data were not normally distributed, did not have homogeneous variances, and were not improved by arcsin transformation. To show the patterns of the data, graphs of the image analysis estimates plotted against the ocular estimates made by the three observers are presented for both years combined. Correlation coefficients are reported for the measures of correlation. For the Kruskal-Wallis tests, mean cover values and nonparametric groupings are reported. The groupings were calculated using a nonparametric multiple comparisons equation (Daniel 1990). Results are reported for all the sampling loci together and for the sampling loci at each site each year.

Reliability was further tested by examining the agreement of the image analysis and ocular estimates at low, medium, and high cover levels to determine if image analysis tended to over or underestimate cover at different cover levels. The image analysis and ocular cover estimates for a single sample locus were divided into cover level groups depending on the mean of the three ocular cover estimates. If the mean of the three ocular cover estimates was <30%, the image analysis and three ocular cover estimates from this sampling locus were placed in the low cover group. If the mean of the three ocular cover

estimates was in the range of 30% to less than 70%, the estimates from this sampling locus were placed in the medium cover group. Similarly, if the mean of the three ocular cover estimates was 70% or greater, the estimates were placed in the high cover group. The data within each cover level group were then compared using a Kruskal-Wallis test (SAS Institute, Inc. 1987). Mean cover values and nonparametric groupings (Daniel 1990) are reported.

Error Level and Sample Size The error levels of the three ocular estimates and the image analysis estimates were determined for each of the 18 plots by year using the equation: $E = s/(\bar{x}\sqrt{n})$ where E = error level, s = standard error of the mean, $\bar{x}$ = sample mean, and n = number of quadrats. In this study, the number of quadrats was 40. The number of quadrats per plot required to estimate leafy spurge cover with a 10% error level at a 95% confidence level was calculated using the equation: $n = (t^2 s^2)/E^2$ where n = number of quadrats, $t = 2.022$ (the two-tailed Student's t-value for 39 degrees of freedom), s = standard error of the mean, and $E = 0.10$ error level (Molloy and Moran 1991, Pieper 1978). High, low, and average error levels and sample sizes are reported along with the Tukey groupings.

Ocular Cover, Image Analysis Cover, and Biomass Comparisons

Biomass and Cover Estimates In 1994, biomass data was collected at the 10 northwest sampling loci in each plot (180 total). At each locus, the leafy spurge within a Daubenmire frame was clipped, placed in brown paper bags, air dried at 35 C for three weeks, and weighed to obtain biomass data. For comparison, prior to clipping,

Observer 3 ocularly estimated the leafy spurge cover and a color photo was taken of the Daubenmire frame at each sampling locus. Observers 1 and 2 ocularly determined leafy spurge cover from each print. Leafy spurge cover was also determined for each print using image analysis.

Biomass Models The leafy spurge ocular cover estimates obtained by the three observers, the image analysis estimates, and the biomass estimates collected from the northwest transects were measured for correlation (SAS Institute, Inc 1987). Linear models were then developed using regression analysis (SAS Institute, Inc. 1987) to relate biomass to leafy spurge cover as estimated by each observer and by image analysis. The correlation coefficients are reported. Graphs show the relationships between the leafy spurge cover estimates and biomass. Linear equations, coefficients of determination (R^2), and *P*-values are reported on the graphs.

Results and Discussion

Precision

Repeatability The comparison of the mean standard deviations from the image analysis method and the ocular method of estimating cover ten times from the ten color prints indicates that image analysis is as repeatable as the ocular method at estimating leafy spurge cover. While there were no statistical differences in mean standard deviations between image analysis and any of the four ocular estimators ($P = 0.5747$), image analysis had a lower mean standard deviation than three of the four observers (Table 2).

Table 2. Comparison of the mean standard deviations of the ten image analysis (IA) leafy spurge cover estimates and ten ocular leafy spurge cover estimates made by each of four observers from ten color prints.

Method	Mean Std. Dev.	Std. Error	Tukey	*P*-Value
	---%---			
Obs 2	4.3650	1.2249	A	0.5747
Obs 3	4.2423	0.9783	A	
Obs 4	3.8946	0.4905	A	
IA	3.2660	0.7824	A	
Obs 1	2.5995	0.6279	A	

Similarly, Stone et al. (1988) found image analysis as repeatable as the ocular photographic grid method at estimating cover of carrots, lettuce, and onions from false color infrared images. The variance of the image analysis method was 0.0572 while that of the ocular grid method was 0.0541. However, Nutter et al. (1993) found image analysis to be less repeatable than the visual method of estimating percent disease severity of dollarspot on bentgrass. Four raters quantified percent disease severity visually from color slides of one m^2 quadrats by dividing the diseased area of the quadrat by the total quadrat area. Another rater quantified percent disease severity from the slides using image analysis. All five raters quantified each slide twice. The researchers determined repeatability among raters by linearly regressing the first and second estimates of each observer. The linear regression R^2-value on the first and second image analysis estimates was 0.63 while the R^2-values of the first and second visual estimates varied from 0.80 to 0.93.

Reliability Graphs of the image analysis estimates plotted against the three observers' ocular estimates show the patterns of the data (Figure 4). Because the points are grouped around the 45° lines, the image analysis estimates appear to generally agree with the ocular cover estimates.

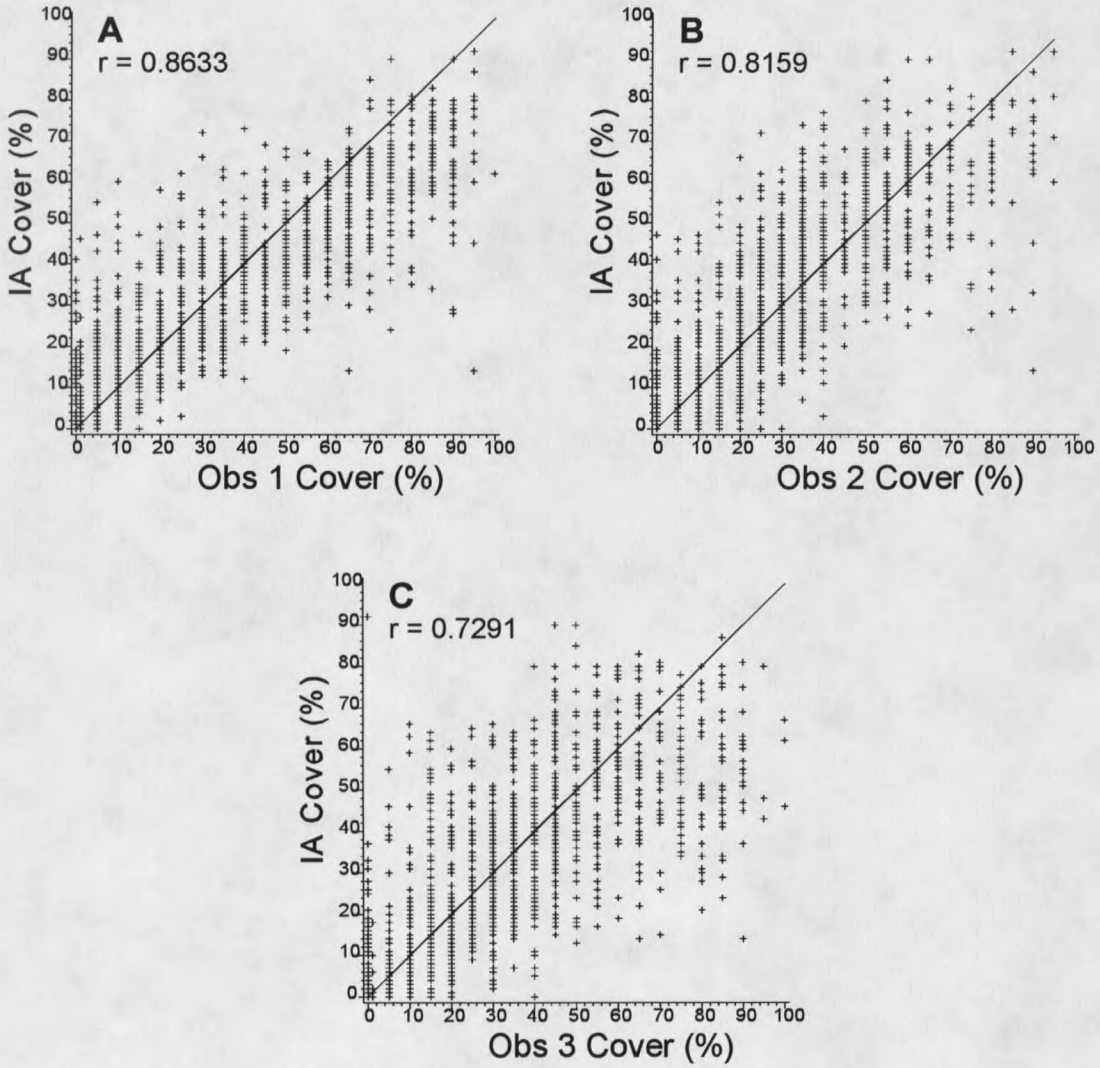


Figure 4. Image analysis estimates of leafy spurge cover in relation to ocular estimates of leafy spurge cover made by Observer 1 from color prints (A), by Observer 2 from color prints (B), and by Observer 3 from field observations (C).

The measures of correlation also indicate agreement between the image analysis and ocular cover estimates. When the image analysis and ocular estimates for both years were correlated (Table 3), the correlation coefficient (r) between the image analysis estimates and Observer 1's estimates was 0.8633, between image analysis' and Observer 2's was 0.8159, and between image analysis' and Observer 3's was 0.7291. The lower correlation coefficient between image analysis and Observer 3 probably resulted from the difference in time in 1993 between the estimates made by Observer 3 in the field and the photographing of the color prints used for image analysis. The highest correlation coefficient occurred between Observer 1 and Observer 2 (r = 0.9205).

Table 3. Correlation coefficients (all *P*-values < .01) between the image analysis (IA) and ocular leafy spurge cover estimates made by three observers with both years and all sites combined.

	Method		
Method	Obs 1	Obs 2	Obs 3
	---------- Pearson Correlation Coefficients ----------		
Obs 2	0.9232		
Obs 3	0.7883	0.7751	
IA	0.8633	0.8159	0.7291

When the image analysis ocular cover estimates from both years were compared using a Kruskal-Wallis test (Table 4), the image analysis estimates did not differ from the ocular estimates of any of the three observers (P = 0.0139). According to the nonparametric groupings, the estimates of Observer 2 differed from those of Observer 3.

However, the mean cover values from the image analysis' and the three observers' estimates were all within 3.7%. Thus, there appears to be no biologically important difference between any of the methods.

Table 4. Mean cover values and nonparametric groupings of the image analysis (IA) and ocular leafy spurge cover estimates made by three observers with both years and all sites combined.

Method	Mean Cover	Std. Error	Grouping	*P*-Value
	-----%-----			
Obs 1	33.8358	0.7290	AB	0.0139
Obs 3	33.1942	0.6215	A	
IA	31.4503	0.5761	AB	
Obs 2	30.1796	0.5793	B	

Measures of correlation conducted for each site each year (Table 5) provide more information. The correlation coefficients between the image analysis and the ocular cover estimates were above 0.62 except in 1993 at Fergus County ($r = 0.3002$). Observers 1 and 2 also had low correlation with Observer 3 at Fergus County (r-values of 0.3629 and 0.2928) which was attributed to the time difference between the estimates made by Observer 3 and the estimates made using image analysis and by the other observers. The Fergus County estimates presumably would have been influenced most by the time difference because of the larger size and quantity of leafy spurge compared with the other sites.

Table 5. Correlation coefficients (all *P*-values < .01) between the 1993 and 1994 image analysis (IA) and ocular leafy spurge cover estimates made by three observers at the Fergus County, Custer County, and Valley County sites.

Year	Method	Site	Method		
			Obs 1	Obs 2	Obs 3
			---------- Pearson Correlation Coefficients ----------		
1993	Obs 2	Fergus	0.8470		
		Custer	0.9630		
		Valley	0.9118		
	Obs 3	Fergus	0.3629	0.2928	
		Custer	0.8213	0.7969	
		Valley	0.7910	0.7455	
	IA	Fergus	0.7634	0.6986	0.3002
		Custer	0.9044	0.8997	0.7291
		Valley	0.7357	0.6760	0.6570
1994	Obs 2	Fergus	0.8536		
		Custer	0.9343		
		Valley	0.9216		
	Obs 3	Fergus	0.8244	0.8542	
		Custer	0.9127	0.9345	
		Valley	0.9134	0.9360	
	IA	Fergus	0.7815	0.7074	0.6285
		Custer	0.9328	0.9045	0.8891
		Valley	0.9206	0.8482	0.8639

Results from the Kruskal-Wallis tests further highlight the relationships between the image analysis and ocular cover estimates at the three sites each year (Table 6). In 1993, the image analysis estimates differed from the ocular estimates of Observer 2 at the Fergus County ($P = 0.0001$) and Custer County ($P = 0.0146$) sites. However, examination of the mean cover values shows that, overall, image analysis was within 7% of Observer 2 at Fergus County and within 3.5% of Observer 2 at Custer County. Thus, the differences are small. In addition, the means of the image analysis estimates at all three sites are between those of the observers, indicating that the observers often differ more from each other than they do from image analysis.

In 1994, the image analysis estimates differed from the ocular estimates of Observer 1 at Fergus County ($P = 0.0001$), from those of Observers 2 and 3 at Custer County ($P = 0.0001$), and from those of Observer 2 at Valley County ($P = 0.0137$) as evidenced by the nonparametric groupings. However, examination of the mean cover values shows that image analysis differed by less than 7% in all cases. Thus, the differences were not biologically significant. Mean cover values determined from the image analysis estimates were lower than the mean cover values determined from the three observers' ocular estimates at Custer ($P = 0.0001$) and Valley County ($P = 0.0137$). Although the means differed by less than 6%, image analysis may tend to slightly underestimate leafy spurge cover compared with the observers.

Table 6. Mean cover values and nonparametric groupings from the 1993 and 1994 image analysis (IA) and ocular cover estimates made by three observers at the three sites.

Year	Site	Method	Mean Cover	Std. Error	Grouping	*P*-Value
			----%----			
1993	Fergus	Obs 3	46.1667	1.1376	A	0.0001
		IA	42.0583	1.0709	A	
		Obs 1	41.9042	1.5307	A	
		Obs 2	35.1458	1.0950	B	
	Custer	Obs 3	31.3909	1.8847	AB	0.0146
		IA	30.7864	1.3620	A	
		Obs 1	30.0182	1.9282	AB	
		Obs 2	27.3409	1.7652	B	
	Valley	Obs 3	33.8875	1.5714	A	0.0610
		IA	32.1333	1.7924	A	
		Obs 1	31.6000	1.1375	A	
		Obs 2	29.3958	1.4293	A	
1994	Fergus	Obs 1	55.6917	1.5587	A	0.0001
		IA	49.7000	1.1918	B	
		Obs 2	46.1042	1.2636	B	
		Obs 3	44.7480	1.1155	B	
	Custer	Obs 3	17.1255	1.0321	A	0.0001
		Obs 2	15.3750	0.8760	A	
		Obs 1	14.2667	1.1075	AB	
		IA	11.4750	0.9296	B	
	Valley	Obs 1	28.6611	1.5080	AB	0.0137
		Obs 2	27.4792	1.1916	A	
		Obs 3	25.6946	1.1370	AB	
		IA	22.9916	1.2402	B	

Since the image analysis leafy spurge cover estimates agree with the ocular cover estimates of most of the observers overall and at each site, the image analysis estimates appear reliable. In all cases, the mean cover values determined from the image analysis estimates are within 7% of the mean cover values determined from any of the three observers ocular estimates. Thus, image analysis appears to estimate leafy spurge cover similarly to the three observers overall and at the three vegetational communities studied.

Other plant science researchers have found image analysis measures to agree with conventional measures. Tagliavini et al. (1993) measured peach root surface areas conventionally and with image analysis and used correlation to compare the measures. The image analysis and conventional measures were highly correlated with an r-value of 0.95. Thomas et al. (1988) used image analysis to measure percent cover of rapeseed from color slides. To determine accuracy, the researchers compared the image analysis and visual estimates of cover with cover measures obtained using a leaf area meter. The image analysis estimates agreed closely with the leaf area meter measures while the visual estimates were higher than the meter measures. Thomas et al. (1988) determined that image analysis estimated rapeseed canopy more accurately than the visual method. Meyer and Davison (1987) used image analysis to determine leaf areas of soybean, sunflower, and velvetleaf. The image analysis estimates of the leaf areas were compared using linear regression analysis with leaf area estimates measured using the electrical optical method. The R^2-values were 0.995 for soybean and 0.993 for sunflower and velvetleaf.

In the present study, the reliability of the image analysis and ocular estimates was further tested by comparing the image analysis and ocular estimates at low, medium, and high cover levels (Table 7). At low cover levels, image analysis estimated leafy spurge similarly to Observers 2 and 3. Observer 1 appeared to slightly underestimate leafy spurge cover ($P = 0.0001$). However, as the mean cover values were all within 5%, there was little overall difference between the cover estimates.

At medium cover levels, image analysis and Observer 3 estimated leafy spurge cover similarly. Examination of the mean cover values shows that the image analysis estimates tended to be lower than those of Observer 1 and higher than those of Observer 2 ($P = 0.0001$). However, the image analysis mean cover value was within 7% of the mean cover values determined from all three observers' estimates. In fact, the observers tended to differ more from each other than from image analysis.

At high cover levels, the image analysis estimates differed from those of all three observers ($P = 0.0001$). An examination of the mean cover values shows that image analysis tended to underestimate leafy spurge cover compared with the observers, with differences ranging from 12 to 22%. These results indicate that image analysis may underestimate cover at high cover values compared to the ocular method of estimating cover. Image analysis may have underestimated leafy spurge at high cover values because image analysis may better account for foliar openings than the observers. The observers might see cover more as the gross crown spread without accounting for any foliar openings (Daubenmire 1959, Kinsinger et al. 1960).

Table 7. Mean cover values and nonparametric groupings of image analysis (IA) and ocular leafy spurge cover estimates made by three observers at low, medium, and high cover levels.

Cover Level	Method	Mean Cover	Std. Error	Grouping	*P*-Value
		----%----			
Low	Obs 3	15.2061	0.4381	A	0.0001
	IA	14.8216	0.4833	A	
	Obs 2	13.1178	0.3336	A	
	Obs 1	10.6590	0.3599	B	
Medium	Obs 1	51.8463	0.6770	A	0.0001
	Obs 3	46.8283	0.6431	B	
	IA	45.0523	0.5757	B	
	Obs 2	42.1315	0.5482	C	
High	Obs 1	84.8387	0.7469	A	0.0001
	Obs 2	76.7742	0.9809	B	
	Obs 3	75.2151	1.2109	B	
	IA	63.4301	1.5046	C	

While the accuracies of the image analysis and ocular estimates were not tested in this study, image analysis appears to be precise. Image analysis was as repeatable as the ocular method of estimating leafy spurge cover and was reliable in most cases. The image analysis estimates were similar to those of at least one of the observers overall, at each site, and at low and medium cover levels. At high cover levels, image analysis underestimated cover compared with the three observers.

Error Level and Sample Size

The error level of a sample is affected by the standard error of the mean which is in turn affected by the standard deviation and sample size. Thus, the error level of a sample can be improved by either reducing the variation of the estimates or increasing the sample size. Table 8 summarizes the error levels for the 18 plots for both years together. There were no differences (P = 0.3555) between average error levels for the image analysis estimates or any of the ocular estimates made by the three observers according to the Tukey groupings. Although there were no statistical differences, image analysis had the lowest average error level (E = 9.93) while Observer 1 had the highest average error (E = 11.71). Interestingly, image analysis had the lowest (E = 3.29) and highest (E = 25.09) error levels found for a plot.

Table 8. Summary of the error levels for the 1993 and 1994 image analysis (IA) and ocular leafy spurge cover estimates made by the three observers at the 18 plots.

	Error Level				
Method	High	Low	Ave	Tukey	*P*-Value
	---------- % ----------				
Obs 1	21.73	4.70	11.71	A	0.3555
Obs 2	18.70	4.84	10.03	A	
Obs 3	17.62	4.64	10.05	A	
IA	25.09	3.29	9.93	A	

Molloy and Moran (1991), using a random process model to examine error levels for the image analysis and ocular grid methods of measuring crop residue cover from prints, found the error level of the photographic grid method to be 27 times higher on average than error for the image analysis method.

A summary of the number of quadrats required per plot to estimate leafy spurge cover with a 10% error at a 95% confidence is reported in Table 9. Image analysis required an average sample size of 10 quadrats. This was fewer (P = 0.0007) than the 17 quadrats required by Observer 1 and the 15 quadrats required by Observer 3 to achieve the same error, but not different from Observer 2's average of 13 quadrats. Image analysis and Observer 2 tied for the lowest number of quadrats needed to achieve a 10% error level in a plot. Image analysis never needed more than 19 quadrats per plot while Observers 1, 2, and 3 required a high of 35, 32, and 38 quadrats, respectively.

Table 9. Summary of the sample sizes needed to estimate leafy spurge cover within a 10% error at a 95% confidence level based on the 1993 and 1994 image analysis (IA) and ocular leafy spurge cover estimates made by the three observers at the 18 plots.

	Sample Size				
Method	High	Low	Ave	Tukey	*P*-Value
Obs 1	35	2	17	A	0.0007
Obs 2	32	4	13	AB	
Obs 3	38	4	15	A	
IA	19	2	10	B	

The required number of image analysis samples reported in this study are supported by Molloy and Moran (1991). These researchers generated a sampling rate graph for the number of one m^2 quadrats required to obtain errors of 5, 10 and 15% at the 95% significance level for different ranges of crop cover measured using image analysis. At cover levels up to 80%, 19 quadrats were sufficient to maintain a 10% error level. However, at cover levels between 80 and 100%, up to 28 quadrats were required. Fourteen quadrats per plot were sufficient to maintain a 15% error level for all levels of crop cover. Thus, to estimate leafy spurge cover within a 10% error level in our study, presumably only 20 quadrats would need to be sampled per plot if image analysis was used to estimate cover. If cover was estimated ocularly, twice as many quadrats would have to be sampled to maintain the same error level.

Biomass Models

Roebertsen et al. (1988) stated that before image analysis can be used to measure biomass, the image analysis values have to be calibrated with biomass measures for the vegetation type. While cover can have a degree of correlation with biomass, separate models must be developed for each species (Bonham 1989). These models are generally of the form $y = a + b[f(x)]$ where y is the biomass estimate, a is a constant, b is the slope of the line, and $f(x)$ is some function of the measured species' cover. The function can be logarithmic or otherwise.

Table 10 contains the correlation coefficients between the image analysis and ocular estimates of leafy spurge cover and the biomass estimates from the northwest

transects. The image analysis and ocular cover estimates had correlation coefficients ranging from r = 0.7919 between Observer 1 and biomass to r = 0.8141 between Observer 2 and biomass. The high correlation coefficients (r-values > 0.79) suggested linear relationships between the image analysis and ocular cover estimates and biomass.

Table 10. Correlation coefficients (*P*-values < .01) between the 1994 image analysis (IA) estimates of leafy spurge cover, the ocular leafy spurge cover estimates made by the three observers, and the leafy spurge biomass estimates from the northwest transects at all sites combined.

	Method			
Method	Obs 1	Obs 2	Obs 3	IA
	---------- Pearson Correlation Coefficients ----------			
Obs 2	0.9345			
Obs 3	0.9180	0.9086		
IA	0.9239	0.9286	0.8889	
Biomass	0.7919	0.8141	0.8082	0.8101

Linear models were generated using regression analysis to relate leafy spurge cover to biomass for image analysis and for the three observers (Figure 5). While the P-values of 0.0001 for the linear models were significant, the overly simple linear models were not consistent for predicting leafy spurge biomass based on the image analysis cover estimates ($R^2 = 0.6563$) nor the ocular estimates made by the three observers ($R^2 = 0.6272$, $R^2 = 0.6627$, and $R^2 = 0.6531$). Logarithmic and polynomial models and arcsine and square root transformations of the cover data did not improve the fit.

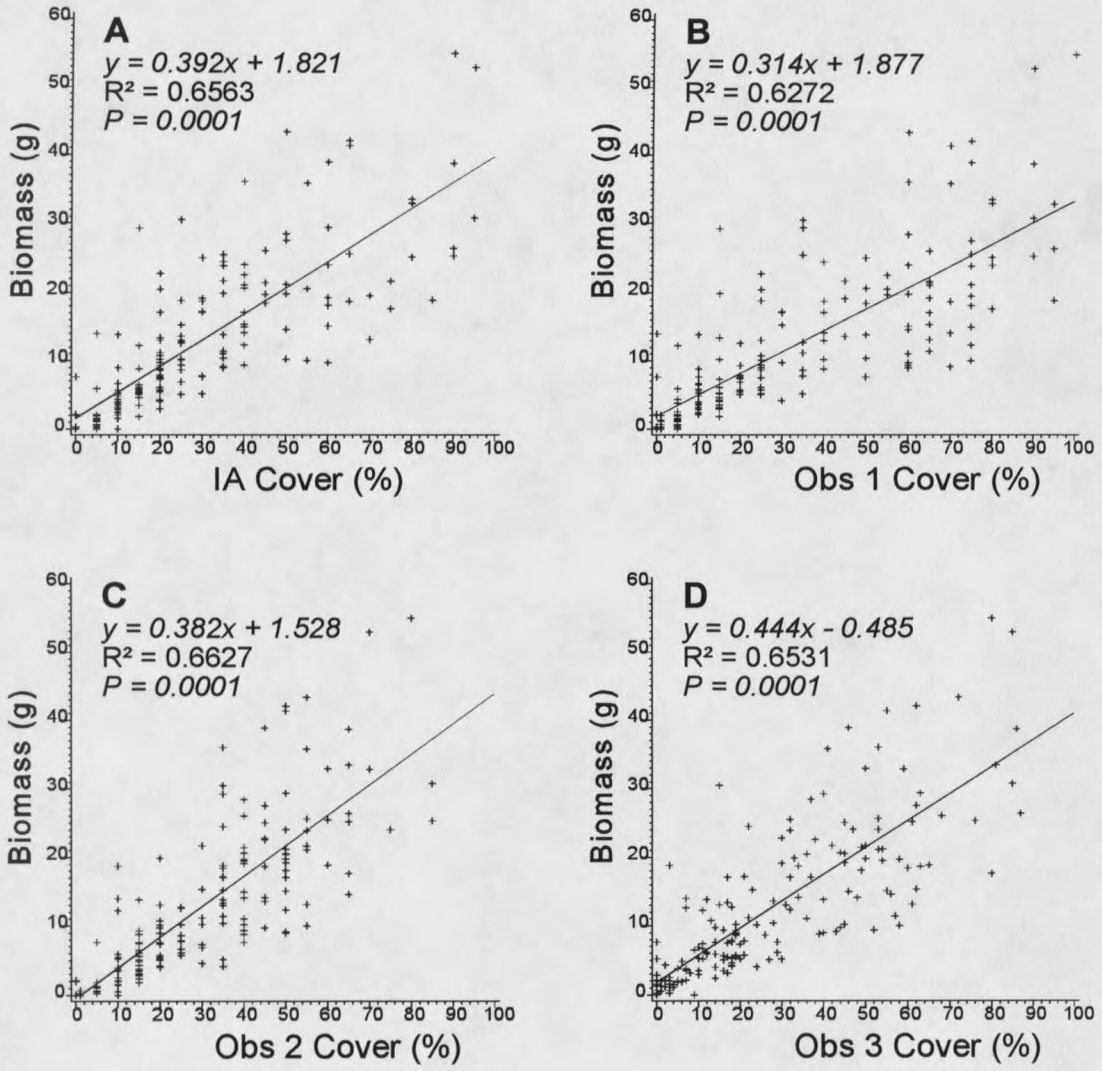


Figure 5. Biomass of leafy spurge in relation to estimates of leafy spurge cover made using image analysis (A), and ocular estimates made by Observer 1 from color prints (B), by Observer 2 from color prints (C), and by Observer 3 from field observations (D).

Addition of a term or terms to the simple linear models such as a leafy spurge height factor might improve the models. Correlation coefficients were lowest (Table 11) between the leafy spurge cover estimates and biomass at the Fergus County site where leafy spurge was the tallest. Since the ocular and image analysis cover estimates in this

study accounted only for crown spread and not overall size or layering of vegetation, the cover estimates may not adequately predict the overall biomass for tall leafy spurge plants.

Table 11. Correlation coefficients (*P*-values < .01) between the 1994 image analysis (IA) estimates of leafy spurge cover, the ocular estimates of leafy spurge cover made by the three observers, and the leafy spurge biomass estimates from the northwest transects at the Fergus County, Custer County, and Valley County sites.

		Method			
Method	Site	Obs 1	Obs 2	Obs 3	IA
		---------- Pearson Correlation Coefficients ----------			
Obs 2	Fergus	0.8315			
	Custer	0.9433			
	Valley	0.9525			
Obs 3	Fergus	0.7735	0.7752		
	Custer	0.9002	0.9315		
	Valley	0.9160	0.9192		
IA	Fergus	0.7956	0.8579	0.7042	
	Custer	0.9051	0.8915	0.8548	
	Valley	0.9147	0.9186	0.8707	
Biomass	Fergus	0.5071	0.6025	0.6086	0.6287
	Custer	0.7715	0.7893	0.8073	0.6718
	Valley	0.8484	0.8730	0.8299	0.8762

Ernst and Kuhn (1989) were successful at developing an exponential model relating image analysis estimates of Benjamin fig leaf area to plant mass while Stone et al. (1984) developed a logarithmic model relating image analysis estimates of crop cover to

crop dry weights. However, our study indicates that models relating image analysis cover estimates to biomass may not be straightforward to develop for all species.

Conclusions

Image analysis was able to separate leafy spurge from the other plant species found at the three sites studied. The image analysis leafy spurge cover estimates appeared reliable when compared to the ocular estimates, although image analysis may underestimate leafy spurge cover at high cover values compared with the ocular method. Image analysis' underestimation at high cover values was attributed to the ability of image analysis to account for foliar openings.

Image analysis was determined to have the same repeatability at estimating leafy spurge cover as the ocular estimators as evidenced by the lack of differences in mean standard deviations. The average error levels of the image analysis and ocular methods did not differ and were within the generally accepted range for field sampling. The number of quadrats required to estimate leafy spurge cover within 10% error at a 95% confidence level differed for image analysis and two of the three observers. In the present study, only 20 quadrats would need to be sampled per plot if image analysis was used to estimate leafy spurge cover while twice as many quadrats would need to be sampled if cover was measured ocularly. Neither the image analysis nor the ocular estimates were reliable predictors of leafy spurge biomass using simple linear models. Logarithmic and polynomial models did not improve the fit. Models relating cover estimates to biomass may not be straightforward to develop.

Image analysis appears to be a useful tool to aid in determining the effects of biological control agents on leafy spurge cover. While the precision of the image analysis method was not markedly different from the precision of the ocular method, fewer quadrats would need to be sampled with image analysis. The color prints from image analysis would also provide a permanent photo record of the study sites. The ability of image analysis to separate leafy spurge from the numerous plant species present at the sites indicates that image analysis might also be able to separate other plant species based on reflectance. Each plant species tends to have a characteristic reflectance signature (Knipling 1970). Guyer et al. (1986) determined that differences in numbers and shapes of leaves could also be used in image analysis to identify individual plant species. Thus, image analysis could be useful not only to measure effects of biological control agents on leafy spurge, but in any study where stress results in reduced total leaf area of a plant community.

To have practical value, image analysis must be rapid and the technological requirements must be generic and relatively inexpensive. While we did not collect data in this study on the time requirements of the image analysis and ocular methods, several observations were made. The color photos needed for image analysis required roughly the same time in the field to collect as did the ocular cover data.

The additional laboratory time required to obtain the image analysis data from color prints has been examined in several studies. McMillan and Schwartz (1993) initially spent a high amount of time acquiring images of bean leaves and performing image analysis on the images. However, after developing a routine, the researchers could obtain

and analyze an image in 20 to 30 seconds. Thomas et al. (1988) found that it took 52 seconds on average to visually inspect a slide and 104 seconds for image analysis. The time required to visually inspect a slide increased as percent cover increased but remained relatively constant for image analysis. Thomas et al. (1988) also noted that visually inspected data are generally recorded on paper. Additional processing time would be needed to enter the visual data onto a computer. Based on our results regarding sample sizes, if only half as many image analysis samples were collected, the time required to collect the image analysis data may actually be lower than for the ocular data.

The technological requirements for image analysis are generic and relatively inexpensive. The equipment needed to perform image analysis currently includes a frame grabber board or scanner, a personal computer, and software. Recently, filmless electronic cameras have been developed which directly transmit digital signals. These cameras may supersede film-based cameras and a scanner or frame grabber would no longer be needed. Inexpensive image analysis software is now available. Experts predict that image analysis programs will continue to become easier to use and less expensive (Grotta and Grotta 1992).

Thus, the application of image analysis appears to have practical value as a measurement tool. The methodology is simple and versatile. Image analysis is automated to a large extent and is relatively rapid. Quantification is shown to be repeatable and reliable and to require fewer samples than the ocular method. The technological requirements are personal computer-based and relatively inexpensive.

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Appendix

List of observed plant species at the three study sites.

Table A-1. List of observed plant species at the Fergus County, Montana site.

Grasses	
Agropyron smithii Rydb.	western wheatgrass
Agropyron spicatum (Pursh) Scribn. & Smith	bluebunch wheatgrass
Bromus inermis Leyss.	smooth brome
Bromus japonicus Thunb.	Japanese brome
Festuca idahoensis Elmer	Idaho fescue
Koeleria pyramidata (Lam.) Beauv.	prairie junegrass
Phleum pratense L.	timothy
Poa pratensis L.	Kentucky bluegrass
Stipa comata Trin. & Rupr.	needleandthread
Forbs	
Achillea millefolium L.	western yarrow
Antennaria DC. sp.	pussytoes
Artemisia ludoviciana Nutt.	cudweed sagewort
Astragalus L. sp.	locoweed
Balsamorhiza sagittata (Pursh) Nutt.	arrowleaf balsamroot
Chrysopsis villosa (Pursh) Nutt.	hairy goldaster
Euphorbia esula L.	leafy spurge
Glycyrrhiza lepidota Pursh	licorice
Lupinus L. sp.	lupine
Medicago lupulina L.	black medic
Medicago sativa L.	alfalfa
Phlox L. sp.	phlox
Psoralea tenuiflora Pursh	slimflower scurfpea
Ratibida columnifera (Nutt.) Woot. & Standl.	prairie coneflower
Selaginella densa Rydb.	clubmoss
Thermopsis rhombifolia Nutt.	round-leaved thermopsis
Tragopogon L. sp.	salsify
Shrubs	
Artemisia frigida Willd.	fringed sagewort
Rosa L. sp.	rose
Symphoricarpos albus (L.) Blake	snowberry

Table A-2. List of observed plant species at the Valley County, Montana site.

Grasses	
Agropyron cristatum (L.) Gaertn.	crested wheatgrass
Agropyron smithii Rydb.	western wheatgrass
Bouteloua gracilis (H. B. K.) Lag. *ex* Steud.	blue grama
Bromus inermis Leyss.	smooth brome
Calamovilfa longifolia (Hook.) Scribn.	prairie sandreed
Koeleria pyramidata (Lam.) Beauv.	prairie junegrass
Schizachryium scoparium (Michx.) Nash	little bluestem
Stipa comata Trin. & Rupr.	needleandthread
Stipa viridula Trin.	green needlegrass
Forbs	
Artemisia ludoviciana Nutt.	cudweed sagewort
Chenopodium album L.	lambsquarters
Chrysopsis villosa (Pursh) Nutt.	hairy goldaster
Dalea purpurea Vent.	purple prairieclover
Euphorbia esula L.	leafy spurge
Medicago sativa L.	alfalfa
Opuntia polyacantha Haw.	prickly pear cactus
Selaginella densa Rydb.	clubmoss
Sphaeralcea coccinea (Pursh) Rydb.	scarlet globemallow
Thermopsis rhombifolia Nutt.	round-leaved thermopsis
Shrubs	
Artemisia cana Pursh	silver sagebrush
Artemisia frigida Willd.	fringed sagewort
Rosa L. sp.	rose
Symphoricarpos albus (L.) Blake	snowberry

Table A-3. List of observed plant species at the Custer County, Idaho site.

Grasses	
Agropyron cristatum (L.) Gaertn.	crested wheatgrass
Agropyron spicatum (Pursh) Scribn. & Smith	bluebunch wheatgrass
Agropyron trachycaulum (Link) Malte	slender wheatgrass
Agrostis stolonifera L.	redtop bent
Bromus tectorum L.	cheatgrass brome
Elymus cinereus Scribn. & Merr.	basin wildrye
Koeleria pyramidata (Lam.) Beauv.	prairie junegrass
Oryzopsis hymenoides (R. & S.) Ricker	indian ricegrass
Poa pratensis L.	Kentucky bluegrass
Poa secunda Presl.	Sandberg bluegrass
Stipa comata Trin. & Rupr.	needleandthread
Forbs	
Artemisia ludoviciana Nutt.	cudweed sagewort
Euphorbia esula L.	leafy spurge
Opuntia polyacantha Haw.	prickly pear cactus
Shrubs	
Artemisia nova A. Nels.	black sagebrush
Artemisia tridentata Nutt.	big sagebrush
Artemisia tripartita Rydb.	threetip sagebrush
Chrysothamnus nauseosus (Pall.) Britt.	rubber rabbitbrush
Purshia tridentata (Pursh) DC.	antelope bitterbrush
Symphoricarpos albus (L.) Blake	snowberry
Tetradymia canescens DC.	gray horsebrush

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