Comparison of Two Computer Techniques and a Visual Technique for the Estimation of Wheat Leaf Consumption by Cereal Leaf Beetle (Coleoptera: Chrysomelidae)[1]

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Abstract Three techniques for estimating wheat foliage defoliation by cereal leaf beetle, *Oulema melanopus* (L.), larvae were evaluated. The techniques were visual estimation, computer estimation with image capture through a flatbed scanner (Lanalyze), and a commercially available video computer image analysis system (CIAS). Both computer-assisted techniques exhibited high levels of repeatability. Both consistently produced errors of less than 3 percent, although each system exhibited different error patterns. The Lanalyze system tended to systematically underestimate actual defoliation of mock leaves, while the CIAS system tended to overestimate actual defoliation. Visual estimators exhibited greater variation among estimates and, on average, greater discrepancies from actual defoliation when compared with the computer assisted techniques. The experience of the observer had a bearing on the accuracy and consistency of visual estimates; more experienced observers had the best accuracy.

Key Words Defoliation estimation, cereal leaf beetle, *Oulema melanopus,* visual estimation, image analysis

Estimating defoliation by pathogens or feeding injury by arthropods is often important in research and pest management. Quantification of foliage consumption by insect pests is critical to developing damage relationships for defoliating pests, but rapid and accurate quantification is often problematic. In the past workers have used chlorophyll spectral absorbance (Herman 1989), overlaid grids (Jensen et al. 1977), photocopy cut-outs (Fullerton 1982, Jensen et al. 1977), pre-exposure tracings of leaves later offered to herbivores (Surgeoner and Wallner 1978), electronic leaf area or plant canopy meters (Kolondny-Hirsch and Harrison 1982, Allison et al. 1995, Helm et al. 1992), combinations of these techniques, and other strategies to measure foliage consumption. The majority of workers, however, have relied on some variation of visual estimation to obtain these data. Indeed, some of the before-mentioned techniques have been calibrated against visual estimates (i.e., Herman 1989). Some

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have used rating scales to define classes of defoliation or injury (Bautista et al. 1984, Barratt and Byers 1992, McNab and Jerie 1993, Allison et al. 1995, Tomkiewicz et al. 1993), while others have estimated the proportion of injured leaf tissue as a percentage (Papp and Mesterházy 1996).

Visual estimation has the advantages of rapidity and portability, but it presents some obvious difficulties. Perhaps the greatest problem associated with visual estimation is variation in experience, skill, accuracy, and precision among observers. Some workers have attempted to reduce this variation by obtaining estimates from multiple observers (Herman 1989) and development of comparative templates to aid estimation (Helm et al. 1992). However, it is difficult to determine if these measures are effective, and very little attention has been paid to assessing the performance of these various approaches.

Estimating foliage consumption of the cereal leaf beetle, *Oulema melanopus* (L.), is a particularly challenging exercise. In small grains, larvae scrape narrow (<1 mm) longitudinal strips from the upper leaf surface. These feeding scars often vary in length and distribution on the leaf. Injured leaves typically maintain entire margins with varying degrees of "window-paned" lower epidermis. Cereal leaf beetle-injured leaves are not amenable to defoliation estimation through overlying grids or cutting and weighing fragments of photocopied images due to the complexity of the injury patterns. Traditional leaf area meters may also be of limited utility; other workers have noted that injury of this type is not well resolved by the sensors of these devices (Peterson et al. 1998).

We conducted studies comparing the performance of two electronic defoliation estimation techniques and a visual estimation technique. Electronic techniques included a flat-bed scanner system and a video image capture and analysis system; visual estimations were obtained from a pool of volunteers with various levels of experience in estimating defoliation. We used both mock leaves of known defoliation amounts and cereal leaf beetle-damaged wheat leaves to assess the performance of each technique.

Materials and Methods

Generation of mock leaf defoliation models. Black rectangular areas of 2.54 × 12.7 cm (32.26 cm^2 total area) were created in Aldus Superpaint® version 3.0 on an Apple Power Macintosh 7200/120. These "leaves" were arranged on a page and varying levels of "defoliation" were created by superimposing white rectangles over the black "leaves." Four models representing 2.00%, 4.00%, 6.25%, and 9.00% increments of injury were constructed using overlays of white rectangular blocks of 0.65, 1.29, 2.02, and 2.90 cm^2, respectively. Defoliation ranges for each model were 0% to 84.00% for the 2.00% model, 0% to 92.00% for the 4.00% model, 0% to 93.75% for the 6.25% model, and 0% to 81.00% for the 9.00% model. The mock leaves were printed on white bond paper with a Hewlett Packard 5P laser jet printer with a fresh toner cartridge at a resolution of 300 by 300 dots per inch (dpi). Dimensions of mock leaves, defoliation increments, and percent defoliation were verified through hand measurement. Each mock leaf of each defoliation model was assigned a number that corresponded to its actual percent defoliation value.

Generation of wheat leaf samples. Wheat leaves injured by cereal leaf beetle larvae were removed from plants grown in the field. Foliage samples were washed with tap water and placed onto paper towels to blot excess moisture. Leaves were then placed onto 35.5 × 21.6 cm sheets of transcription paper (sticky on one side) (Medical Arts Press, Minneapolis, MN) parallel with each other and perpendicular to the long page edge. The leaves were spaced 3 mm apart so that no portion of one leaf edge intersected another leaf edge (because leaf overlap will result in incorrect analysis). A 35.5 × 21.6 cm PET-G Vivak® 0.51 mm thick polyvinyl sheet (AIN Plastics Co., Greensboro, NC) was placed over the leaves to prevent the transcription paper from adhering to the photocopier deck. The darkness scale of the photocopier was adjusted so that defoliated regions of leaves would be white and intact regions of leaves would be black on the finished photocopy. Fifty photocopied images of leaves that ranged from the lowest to the highest levels of injury observed in the field were used to evaluate the performance of the three estimation systems.

Lanalyze technique. A Hewlett Packard Scanjet 4C flatbed scanner connected to a Gateway 2000 P5-133 computer with a custom software program ("Lanalyze") was used to estimate defoliation for both the mock leaves and the photocopied wheat leaves. As many as 10 leaves were placed on the scanner deck for each scanner pass. Initial scans were adjusted to sharp black and white 100 by 100 dpi images by the Hewlett Packard image acquisition software and finished scans were completed by Corel Photoshop software version 5.0. Scanned images were saved to diskette with a .pcx extension (Windows paintbrush) so the file was accessible to the Lanalyze software. Five scans of each leaf from random locations on the scanner deck were performed to generate means and standard errors, which were used to evaluate Lanalyze performance with other defoliation estimating techniques.

CIAS technique. A computer image analysis system ("CIAS" CID Inc, Vancouver, WA) composed of a Canon Re-350 Video Visualizer, color monitor, a Targa+ image digitizing board and software was used to estimate defoliation for both the mock leaves and the photocopied wheat leaves. Each leaf was placed on the visualizer light table with overhead florescent lighting, and a grayscale image was captured. Grayscale thresholds were set to differentiate between intact foliage and defoliated areas, and percent defoliation was gathered in a two-phase measurement operation (the areas of both the missing and intact foliage were individually calculated). The data were transferred to a spreadsheet for summarization. Each leaf was measured five times in random order and was moved about the visualizer deck between measurements.

Visual rating technique. Visual percent defoliation ratings of both the mock leaves and photocopies of the cereal leaf beetle injured leaves were provided from eight different individuals. These volunteers ranged in general entomological experience from 1 month to over 30 years; our least experienced volunteers had never previously estimated defoliation, while our most experienced volunteers had done this kind of work many times in many different pest-crop systems. Each volunteer was given a data sheet and the mock leaves randomly assorted; each mock leaf was identified by a code number. The volunteer was instructed to estimate the percent of each figure occupied by white space to the nearest percentage point. The procedure was repeated for the photocopies of cereal leaf beetle-injured wheat leaves. Each volunteer was requested to give a single estimate of each leaf and not to compare leaves. Multiple estimates of individual leaves were not pursued to avoid volunteer fatigue.

Statistical procedures. The estimatible error for the mock leaves in the four models was determined by subtracting defoliation estimates provided by each technique from the known defoliation values. Estimatible error was analyzed to evaluate the accuracy and repeatability of the three defoliation rating techniques. Additionally, the mock leaf models were divided into low (0% to 33.3%), middle (33.4% to 66.6%), and high (66.7% to 100%) ranges to examine the degree of within model estimatible error among the three rating techniques.

Mean defoliation estimates for the cereal leaf beetle-injured wheat leaf photocopies from the three rating techniques and eight individual visual defoliation estimators were compared through linear correlation analysis. Defoliation estimates of the CIAS and visual techniques were regressed on Lanalyze estimates and with each other. In addition, individual visual estimators representing the apparent extremes in agreement with Lanalyze and CIAS (the two most closely agreeing and the two most divergent) were regressed individually against the two computer techniques.

Mock leaf data were subjected to analysis of variance using PROC GLM of SAS ($P < 0.05$) (SAS Institute 1990). Wheat leaf defoliation estimates from the three rating techniques were compared through PROC CORR and regressions were generated by PROC REG (SAS Institute 1990). All means and standard deviations were generated by PROC MEANS (SAS Institute 1990).

Results and Discussion

Mock leaf analysis. Highly significant rating technique, defoliation model, model range factors and interactions between these effects were detected in the analysis of estimatible error (Table 1). The significance of the rating technique by defoliation model by model range interaction indicated that the rating techniques estimated defoliation differently among the three ranges within each of the four defoliation models (F = 3.94; df = 12, 1620; P = 0.0001) (Fig. 1). Lanalyze overestimated defoliation in the middle and high ranges of the 9.0% model while defoliation was underestimated for all other model and range combinations. Estimatible error for the Lanalyze technique exceeded 2.0% in the high range of the 2.0%, 4.0% and 6.25% models; however, these errors remained below 3.5%. The CIAS technique overestimated defoliation in all but the high range of the 2.0%, 4.0% and 6.25% models.

Table 1. ANOVA comparing CIAS, Lanalyze, and visual estimation of defoliation in mock leaves across four defoliation increment models and three ranges of defoliation within each model

Factor	df	F	Prob.
Estimation Technique	2	130.54	0.0001
Defoliation Model	3	24.37	0.0001
Defoliation Range	2	32.41	0.0001
Technique X Model	6	34.30	0.0001
Model X Range	6	5.59	0.0001
Technique X Range	4	15.76	0.0001
Technique X Model X Range	12	3.94	0.0001
Error	1620		

Analyses performed using Type III sums of squares (Proc GLM, SAS Institute 1990).

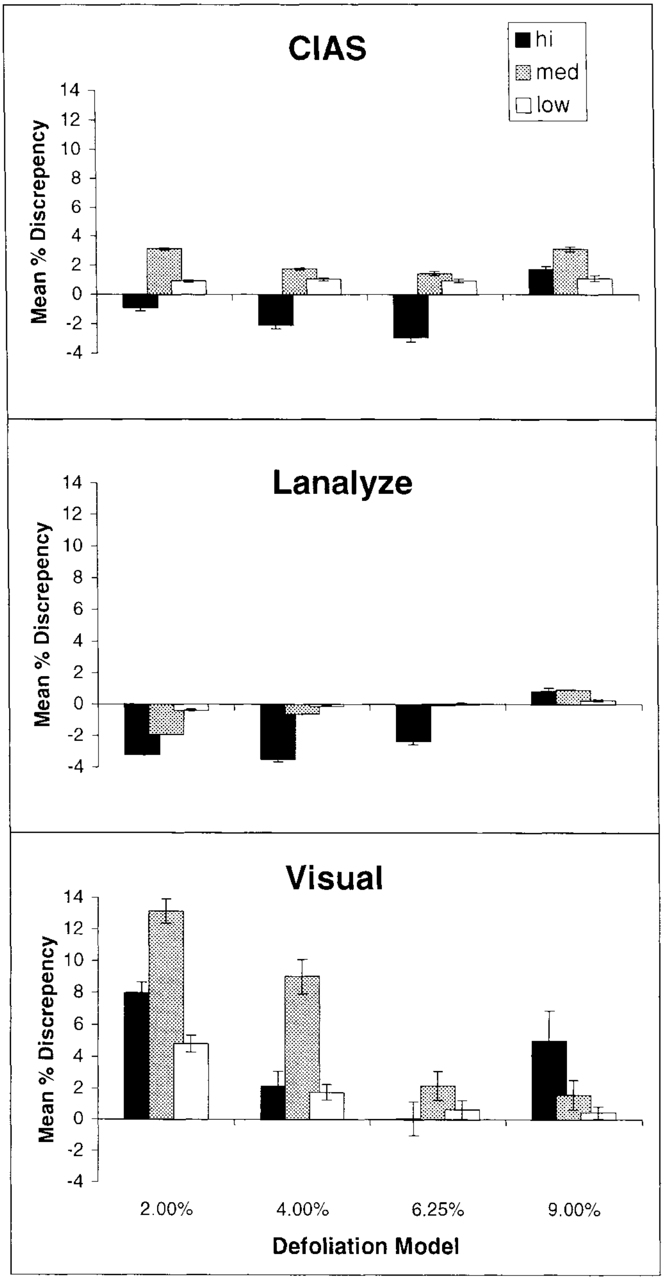

Fig. 1. Discrepancies in defoliation estimations in three defoliation estimation techniques using mock leaf models.

Estimatible error for the CIAS technique exceeded 2.0% in the middle range of the 2.0% and 9.0% models and the high range of the 4.0% and 6.25% models, but the error was not greater than 3.15%. The visual technique overestimated defoliation for all model and range combinations. The visual technique produced levels of estimatible error between 2.0% and 13.14% within all ranges of the 2.0% model, the middle and high range of the 4.0% model, the middle range of the 6.25% model and the high range of the 9.0% model. Standard deviations associated with estimatible error means for all model and range combinations fell between 0.07% and 1.11% for the Lanalyze technique and 0.57% and 1.63% for the CIAS technique. The standard deviations of the estimatible error means generated by the visual technique were greater than the two computer techniques and fell between 2.20 and 9.10.

Visual estimators overestimated defoliation across the models, and the discrepancies were larger with smaller increments of defoliation. Based on these studies, visual estimators could be expected to perform with less precision with smaller units of foliage removal, while both computer estimation techniques could be expected to perform with approximately the same accuracy and precision they exhibited across the tested range of defoliation increments. Because the units of defoliation injury caused by cereal leaf beetle feeding are often substantially smaller than the injury units of the 2% model examined here, and the distribution of damage on affected leaves is less uniform than in the mock leaves, visual estimation of defoliation caused by cereal leaf beetles could potentially be proportionately less accurate than it was with these mock models.

Visual estimation produced errors as great as 14% of total area, while both computer techniques consistently produced errors of less than 2% of total area. Visual estimators with more experience (i.e., estimators 6 and 7 in Fig. 2) produced estimates with lower discrepancies than inexperienced volunteers (estimators 3 and 4 in Fig. 2).

Cereal leaf beetle injured wheat leaves. When data were averaged over the 50 leaves, mean defoliation was 20.55%, 18.80% and 13.10% for the visual, CIAS and Lanalyze techniques, respectively (Table 2). The range between minimum and maximum leaf defoliation estimated means, the 50 respective means and their associated standard deviations were greater in order of presentation for the visual, CIAS and Lanalyze techniques. Additionally, the slopes of lines from regressions of the standard deviation against leaf defoliation means were greater for the visual technique (0.29X) than for the CIAS (0.015X) and Lanalyze (0.006X) computer techniques. This suggests that visual estimator error tended to increase as defoliation increased compared to the computer systems.

Highly significant positive correlation coefficients were detected when leaf defoliation means of Lanalyze were compared with CIAS means ($r = 0.97$; $P = 0.0001$) and visual means ($r = 0.98$; $P = 0.0001$). Additionally, CIAS means were highly correlated with visual means ($r = 0.96$; $P = 0.0001$). High coefficients of determination were observed from linear regression analysis between Lanalyze and CIAS techniques ($y = 1.87 + 1.29x$; $R^2 = 0.95$), Lanalyze and visual techniques ($y = 1.12 + 1.48x$; $R^2 = 0.96$), and CIAS and visual techniques ($y = 0.13 + 1.09x$; $r^2 = 0.91$). An examination of the correlation coefficient matrix for the three techniques (Table 3) indicates that the two least experienced volunteers (volunteers 3 and 4) produced estimates that had the least agreement with the computer techniques.

The computer-based systems performed on average, comparably. The Lanalyze system tended to systematically underestimate actual defoliation in the mock leaves

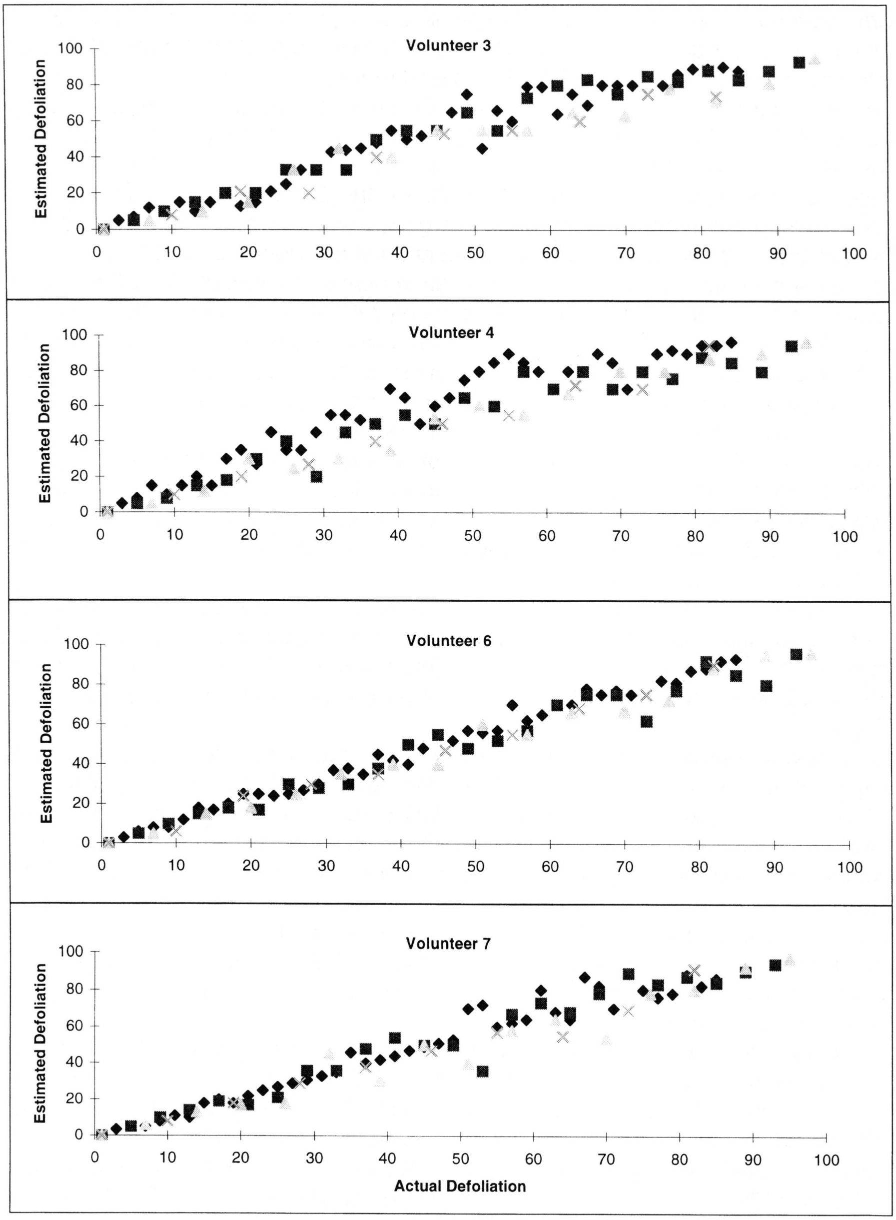

Fig. 2. Relationship between visual estimated defoliation and actual defoliation of mock leaves for four observers with a range of experience.

Table 2. Summary statistics for estimates of wheat leaf defoliation by three estimation techniques

Technique	Mean	Standard deviation	Minimum	Maximum
Lanalyze	13.10	8.59	0.04	34.6
CIAS	18.80	11.41	0.32	43.5
Visual Mean	20.56	12.97	1.38	51.8
Volunteer 1	17.18	15.71	1.0	60.0
Volunteer 2	32.92	20.34	2.0	80.0
Volunteer 3	12.10	7.12	1.0	30.0
Volunteer 4	14.34	13.30	0	55.0
Volunteer 5	17.50	12.16	1.0	50.0
Volunteer 6	24.88	16.96	2.0	75.0
Volunteer 7	25.94	13.91	1.0	58.0
Volunteer 8	19.60	10.55	0	45.0

Table 3. Correlation matrix for wheat leaf defoliation estimates by three techniques

Technique/ Volunteer	Lanalyze	CIAS	Visual, mean
Lanalyze	—	0.972	0.982
CIAS	0.972	—	0.955
Volunteer 1	0.941	0.909	0.954
Volunteer 2	0.920	0.884	0.954
Volunteer 3	0.902	0.850	0.895
Volunteer 4	0.894	0.871	0.937
Volunteer 5	0.965	0.945	0.966
Volunteer 6	0.895	0.878	0.929
Volunteer 7	0.944	0.927	0.944
Volunteer 8	0.958	0.946	0.950

All correlation coefficients significant at $P = 0.0001$.

while the CIAS tended to overestimate actual defoliation; however, the discrepancies were always less than 3.5% and in most cases were below 2% total area. These relationships were fairly consistent across models. The mock leaf data would, therefore, suggest that the actual defoliation values for the cereal leaf beetle-damaged leaves fell between the estimates generated by the two computer-based systems. Visual estimates for the wheat leaves were consistently higher than those from either computer technique, suggesting that in this instance, as with the mock leaves, visual estimators tended to systematically overestimate (by a wider margin than the computer based systems) actual defoliation.

The small discrepancies in measurements we observed with the computer techniques could be associated with several aspects of the procedures. Binarization, how an imaging device assigns a value (in these cases either "black" or "white") to individual pixels in a grayscale image that may not be entirely black or white, was likely involved to some extent with the CIAS system. Pixelation occurs when the resolution of the measuring device is not great enough to adequately describe curves or other changes in the captured image; pixelation probably played a minor role in the errors generated by both systems. Binarization and pixelation could be expected to have greater influence under conditions that generate more "edge" in an image. The exposure settings used to capture an image (brightness, contrast, etc.) could also have had a bearing on pixel classification. Finally, and especially in the case of the CIAS system, minute variations in the lighting environment of the video camera at the time of image capture may have influenced pixel classification from one image to the next.

Visual estimation has the obvious advantages of rapidity and low cost. In these studies, total processing time for an individual estimate was substantially lower than for either of the computer techniques. Further, estimates can easily be made in the field, and nondestructive sampling is possible. However, the discrepancies produced by some visual estimators in this study would have spanned much of the range of defoliation commonly observed in the field, suggesting that observer variability could compromise data if not carefully considered. Observer fatigue is also a potentially significant problem. Training visual observers may improve the estimates they generate, and our data demonstrate that experienced estimators do produce more accurate estimates. Performance of visual estimators might also be improved through the use of reference cards or other visual aids. Although visual estimation by experienced estimators may have advantages in terms of cost and time over computer-based systems, successful implementation may require extensive training, and visual estimation may still not provide resolution fine enough for some distinctions. For the specific problem at hand, visual estimation may not provide sufficient resolution for cereal leaf beetle management decisions based on defoliation estimates.

Although the two computer-based systems used in these studies performed comparably, there are advantages and disadvantages associated with each. The Lanalyze system is less expensive in terms of equipment and software costs than the CIAS system. Lanalyze requires a PC, imaging software, and a flatbed scanner; these can be obtained for approximately $2,000 or less. This system also requires access to a photocopier. Several leaves can be done essentially simultaneously, and total processing time, including sample preparation and photocopying, is similar to that of the CIAS system. The program used to calculate defoliation from captured images is available free from R. A. Ihrig. However, while this system is relatively economical, it does require photocopier processing of collected leaf samples prior to measurement, and it is relatively inflexible with respect to the resolution of captured images and the dimensions of candidate samples.

The CIAS system offers great flexibility in image capture and manipulation. Images can easily be captured from fresh leaf samples and the resulting images can be readily enhanced to improve damage identification. Resolution and magnification can be changed, and captured images can be combined with or spliced to other images. The software driving this system is extremely powerful and offers a wide range of measurement options, and macros can be developed for complex measurement processes. Processing time over many samples seems to be similar to the scanner based system; the total time per sample for both methods ranged between 30 and 50 seconds (C. E. Sorenson and R. A. Ihrig, unpubl. data). Samples are processed individually with this system. However, while the CIAS system used in these experiments is extremely powerful, it represents a substantially greater monetary investment than the Lanalyze system; the total cost for the system as used in these studies approaches $10,000. It also must be noted that while both computer systems produced estimates substantially closer to actual defoliation than visual estimation, these estimates still had a component of error, albeit small.

Visual estimation of cereal leaf beetle defoliation should be reserved for instances where fine classification of damage increments is not needed. If time and financial constraints allow, computer assisted defoliation estimation is preferable to visual estimation.

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