Quantification of Foliar Plant Disease Symptoms by Microcomputer-Digitized Video Image Analysis

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ABSTRACT

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The area of leaf lesions caused by *Alternaria solani* on tomato and *Ascochyta pteridium* on bracken fern, as well as the area of mycelia of *Microsphaera alni* on sycamore and marginal leaf necrosis of California buckeye, were quantified by using computer-controlled video digitizing hardware. Leaves under fluorescent lamps equipped with a red (λ = 620–700 nm) filter were scanned with a black-and-white video camera. BASIC and machine language programming in an Apple II computer equipped with a video analog-to-digital converter were used to locate and digitize each of 16,232 individual pixels into 64 values of grey. The digitized picture elements were grouped into three magnitude categories corresponding to black background, healthy, and necrotic tissue. Algorithms utilizing a correction for background variation allowed reproducibility to ±0.8% in successive measurements. Measurements of individual leaves, application of correctional algorithms, and printing and storage of the data required 4.1 sec. Estimated total and necrotic leaf areas differed from areas measured with a planimeter by <1.2 and 2.0%, respectively.

Quantitative assessments of either foliar disease incidence or severity is important in any study relating disease incidence or severity with crop losses. Accurate assessment of diseased plants will also further the development of quantitative models of plant disease epidemics. Remote sensing has proven valuable primarily for assessing disease incidence (2,4,11,12). Analysis of false-color infrared photographs with microdensitometers or electronic scanners has allowed quantification of disease incidence, usually of entire fields or groups of fields (3,6,8,10,12,13). In contrast, disease severity, particularly of individual leaves or plants, has been evaluated almost exclusively by visual examination. Horsfall and Barratt (5) have pointed out the limitations of visual assessment of plant disease symptom severity, citing the Weber-Feckner law, which states that visual discrimination is a function of the logarithm of the intensity of the stimulus. They noted that the eye can accurately assess only very low or very high levels of disease. Pictorial assessment keys illustrating progressive increases in disease severity have been developed for a number of plant diseases (7). Such keys aid visual analysis of disease severity, but are subject to visual limitations as well as variability due to observer subjectivity. Also, a separate key must be constructed for each disease. Visual estimates of disease obtained by using keys constructed for the evaluation of a leaf spotting fungus causing highly irregular lesions on a plant with highly irregular leaves (eg, *Ascochyta pteridium* Bres. on bracken fern [*Pteridium aquilinum* L. Kuhn][14]) were subject to a high degree of uncertainty and thus variability.

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The purpose of this study was to develop and evaluate a versatile, inexpensive, accurate, and rapid automated system for quantifying the severity of foliar plant disease symptoms by utilizing recent advances in computer science and electronics. A preliminary account of this study has appeared (9).

MATERIALS AND METHODS

The automated disease assessment device. Total leaf area and area of necrotic tissue were calculated following analysis of black-and-white video images of detached leaves or potted plants. Plant material was placed on a black velvet surface in a light-proof box and illuminated with red light ($\lambda = 620$–700 nm) to minimize visible fluorescence and to enhance brightness contrast between green (red-absorbing) and nongreen plant tissue. A continuous video image of plant tissues was generated with a black-and-white video camera (RCA model TC 2011, RCA/Closed-Circuit Video Equipment, Lancaster, PA 17604) equipped with an 8-mm, wide-angle video lens. Camera performance was optimum when the camera was operated with automatic gain control off, with the peak-averaging controls responsive only to average scene brightness, and with the gamma controls set to reduce contrast in the darker areas of the picture. The camera had a resolution of 254 vertical and horizontal lines, which produced an image composed of 64,516 picture elements (pixels).

To assess leaf area and necrotic tissue area, video images were digitized and the data were processed by a microcomputer. Individual picture elements in the video image were located, and pixel intensity was quantified by using a DS-65 Digisector video digitizer (The Micro Works, Del Mar, CA 92014) interfaced with an Apple II Plus microcomputer with 49,152 bytes of RAM memory (Apple Computer Inc., Cupertino, CA 95014). The DS-65 video digitizer converts the analog signal of a given pixel to a digital value with a resolution of 64 levels of grey (from 0 [black] to 63 [white]). The variable-contrast adjustment on the DS-65 peripheral was set at its minimum for optimum performance in this application. Individual pixels at predetermined locations in the video image could be addressed and digitized under either BASIC or Assembly language program control by the Apple II computer (Fig. 1). Thus, either all or a predetermined subset of the pixels comprising a video image could be selected for further analysis. An Assembly language program was used to allow rapid location and retrieval of digitized pixel intensities for routine operation of the disease symptom assessment device.

Sorting of pixels of a video image according to magnitude (shade of grey) permitted the estimation of leaf area and area of necrotic lesions corresponding to a black background, healthy green leaf tissue, or necrotic leaf tissue. Pixels of varying intensities were grouped into one of three magnitude categories. Pixels from images of a black velvet background material had magnitudes generally <6 (Fig. 2). Most pixels from images of healthy green leaf tissue had magnitudes >6, but <51, while most pixels from images of necrotic leaf tissues had magnitudes >51 (Fig. 2). A regular array containing 16,232 individual pixels of an entire video image could be located, digitized, and categorized in 3.7 sec. The fraction of total pixels grouped in each magnitude category (black background, healthy, or necrotic leaf tissues) yielded an initial approximation of the proportion of the video image corresponding to these three objects. Factors contributing to variance in the area determinations include light intensity, camera aperture and performance, and the type of plant tissue (ie, the shade of green of the healthy leaf) being examined.

Corrections to the initial area approximations were possible by separately estimating the fraction of pixels incorrectly assigned to one category from another. Prior to analysis of a given plant species, a small area of the video image delimited by 992 pixels occupied exclusively by healthy leaf tissue of the same species was digitized and categorized into the three magnitude categories. This analysis yielded an estimate of the small portion of pixels corresponding to healthy leaf tissue that would be grouped mistakenly in the category corresponding to the black velvet background (Figs. 1 and 2). Similarly, another initial analysis of approximately 9,000 pixels corresponding to healthy leaf tissue of a given plant species yielded an estimate of the small fraction of pixels that would be mistakenly grouped into the category corresponding to necrotic leaf tissue (Figs. 1 and 2). An area containing 992 pixels in the corner of the video image devoted exclusively to black background also was automatically digitized prior to and following each analysis of an entire video image containing both healthy and necrotic leaf tissue on a black background (Fig. 1). An analysis of this background at each determination allowed estimation of the small fraction of pixels corresponding to black background that would be mistakenly grouped in a category corresponding to healthy leaf tissue.

Determination of the areas of healthy leaf and necrotic leaf tissues can be summarized in the following five steps. First, initial estimates of the background, healthy leaf, and necrotic leaf areas were obtained by digitizing a regular portion of the video image and grouping the values for the individual pixels into three magnitude categories. Second, correctional algorithms under BASIC

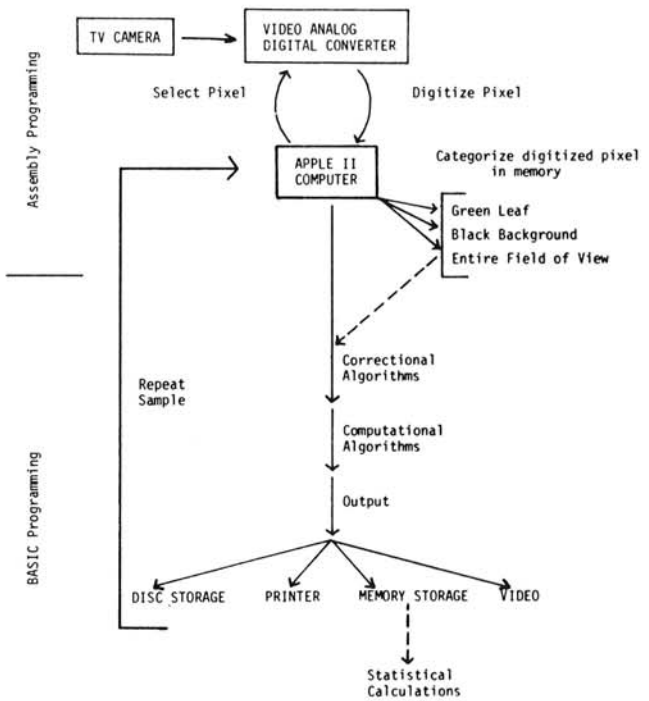

Fig. 1. Flow chart of the operation of the automated disease assessment device.

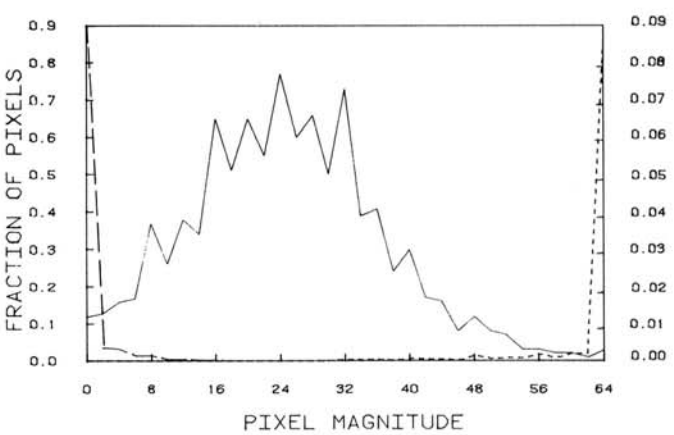

Fig. 2. Distribution of pixel magnitudes of video images of a black velvet background ——, and necrotic tomato leaves infected with *Alternaria solani* ---- (left scale); or of healthy bean leaves —— (right scale).

programming control utilizing initially determined estimates of the portion of healthy leaf categorized as either black background or necrotic leaf tissue, as well as estimates of black background categorized as healthy leaf tissue, were employed to yield final corrected estimates of the total video image corresponding to black background, healthy leaf tissue, or necrotic leaf tissue (Fig. 1). Third, computational algorithms, including relationships of the area observed by the video camera as a function of distance, were utilized to calculate areas of leaves or single or coalesced necrotic lesions (Fig. 1). Availability of a value for average lesion size also permitted calculation of the number of lesions per leaf. Fourth, final results of a given sample were displayed on a second video monitor, printed, and stored in the memory and a floppy-disk subsystem of the Apple II computer (Fig. 1). Finally, statistical calculations utilizing stored data were automatically computed in real time under BASIC programming control following completion of each experiment (Fig. 1). A single determination of leaf area and necrotic leaf area including estimation of error and correctional and computational algorithms required 4.1 sec. The assessment system for a given disease could be set up and 100 leaves processed within 50 min.

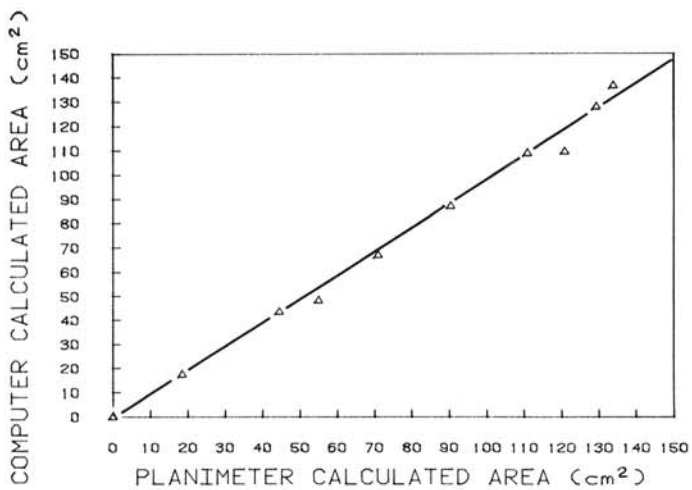

Fig. 3. Relationship between total leaf area of Pinto bean as determined from leaf tracings with a planimeter (abscissa) and as computed from video image analysis (ordinate). The line drawn represents the linear regression $y = 0.996x - 0.44$; coefficient of determination = 0.99 ($P < 0.001$).

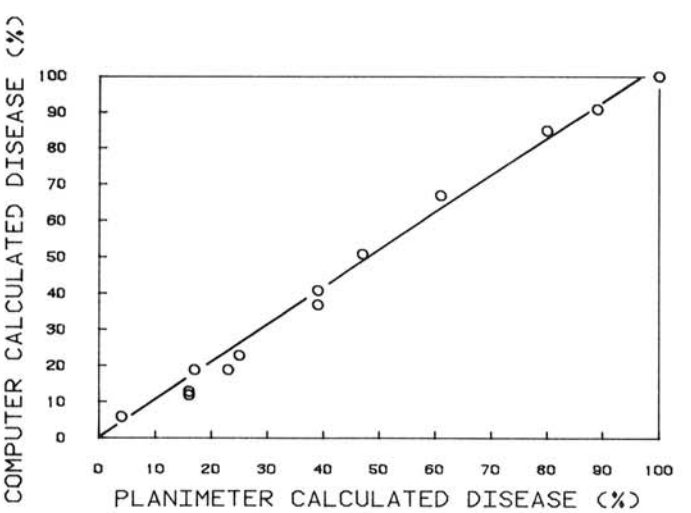

Fig. 4. Relationship between percent of California buckeye leaf area necrotic as determined from leaf tracings with a planimeter (abscissa) and as computed by video image analysis (ordinate). The line drawn represents the linear regression $y = 0.99x - 1.90$; coefficient of determination = 0.97 ($P < 0.001$).

Assessment of plant disease severity. The leaf symptom severity of four separate diseases was evaluated with the automated disease assessment device. In each case, from 10 to 20 individual leaves representing a range of necrosis from 0 to nearly 100% were selected for analysis. Three-week-old tomato (*Lycopersicon esculentum* L.) plants were sprayed with an aqueous suspension of conidia of *Alternaria solani* (Ell. & G. Martin), placed in a dew chamber at 20 C for 2 days, and then placed on a greenhouse bench for 4 days to allow necrotic lesion development. One-month-old fronds of bracken fern (*Pteridium aquilinum* L. (Kuhn)) were inoculated with an aqueous suspension of conidia of *Ascochyta pteridium* Bres., placed in a dew chamber at 18 C for 2 days, and then placed on a greenhouse bench at 21 C for 10 days to allow necrotic lesion development. Three-month-old leaves of sycamore (*Platanus racemosa* Nutt.) naturally infected with *Microsphaera alni* DC. ex Wint. and California buckeye (*Aesculus californica* (Spach) Nutt.) with marginal leaf necrosis were collected from trees near Berkeley, CA. Total leaf area of healthy primary leaves of Pinto bean (*Phaseolus vulgaris* L.) also was computed.

Leaf and lesion areas were measured manually for comparison with the automated disease assessment device. Individual leaves of each plant species were photographed on Plus-X Pan film through a red filter. Total and necrotic leaf area of each leaf was obtained by tracing the entire leaf and necrotic lesions on enlarged photographs with a planimeter (Electronic Graphics Calculator, model E.G.C., Numonics Corporation, Lansdale, PA 19446). The mean total and necrotic areas determined from three tracings of each photograph were considered the true disease severity for each leaf. Each leaf also was viewed with the black-and-white video camera and the video image was analyzed as above. The mean total area or fraction of leaf area diseased from three determinations with the automated disease assessment device was determined within 1 hr of photographing each leaf. Visual estimates of severity of infection by *Ascochyta pteridium* on bracken fern were made by comparison with a 10-stage disease severity key with known proportions of disease from 3 to 97% depicted on bracken fronds.

RESULTS

Automated disease assessment device performance. The discrimination of necrotic tissue from healthy leaf tissue and leaf material from a black background in digitized video images was enhanced utilizing their differential absorption of red light (Fig. 2). Without the illumination of leaves with red light, many necrotic lesions did not appear sufficiently brighter than healthy leaves to allow differentiation by digital image analysis.

Differences in pixel magnitudes were sufficient to enable easy grouping of digitized pixels into three categories corresponding to background, and healthy and necrotic leaf tissue. Typically, less than 5% of the pixels corresponding to any one of these three objects were grouped into categories corresponding to either of the other two objects using pixel magnitudes of 6 and 51 as brightness thresholds (Fig. 2). Therefore, reasonable estimates of total and necrotic leaf area could be obtained by a simple grouping of pixel magnitudes into one of three brightness categories and determining the fraction of the total pixels in each category.

Fluctuations in light intensity and camera performance as well as differences in green pigmentation in healthy leaves because of species or cultural differences required that automatic corrections be made for these differences to ensure reproducibility of determinations. Correctional factors obtained from initial determinations of the distribution of pixel magnitudes for healthy leaves and automatic determination of pixel magnitudes of the dark background in each determination greatly reduced variability in system performance. The average extreme determination of total or necrotic leaf area differed from the mean of five successive observations of a given leaf by only 0.8%.

Increases in system performance with increasing numbers of pixels analyzed was associated with progressively longer execution times. Analysis of approximately 25% of the total pixels in each

video image was chosen as an acceptable compromise on system performance and speed. Only small improvements in the resolution and reproducibility of the automated disease assessment system were obtained by increasing the number of pixels digitized and categorized from the 16,232 routinely analyzed.

The automated disease assessment device performed well on intact potted plants as well as on detached leaves. Average disease severity could be determined on several leaves of potted plants simultaneously, with error approaching that for single detached leaves if illuminated with a uniform diffuse red light source.

Accuracy of the automated disease assessment device. The area of Pinto bean leaves calculated from video image analysis very closely matched the actual leaf area as determined with a planimeter (Fig. 3). The accuracy of assessment of leaf area was independent of leaf size (Fig. 3). The average error in calculation of Pinto bean leaf area from digital video image analysis was approximately 1.2%, assuming that the error in determination of actual leaf area by planimeter was negligible compared to determinations from video image analysis. Leaf areas of other plants with highly irregular leaves such as bracken fern or tomato were also computed from video image analysis with an accuracy of greater than 98%.

The area of necrotic lesions on leaves of four different plant species calculated from video image analysis very closely matched actual necrotic area as determined with a planimeter (Figs. 4–7). These four diseases involved hosts of different leaf size, shape, pigmentation, and veination, as well as lesion or fungal colony size, shape, and pigmentation. Thus, the programming and optical procedures used in construction of the automated disease assessment device were sufficiently flexible to permit assessment of diseased portions of highly variable leaves and lesion types. The area of uniformly pigmented confluent marginal necrosis on relatively simple California buckeye leaves calculated from video image analysis was highly correlated with the actual area as measured with a planimeter (Fig. 4). Estimates of disease severity using visual assessment keys agreed reasonably well with actual areas of necrotic lesions with such large regularly shaped lesions on the simple leaves of this species. The area of mycelial colonization by *M. alni* on sycamore leaves calculated by video image analysis also closely matched the actual areas as measured with a planimeter (Fig. 5). Leaf surface mycelial colonization of *M. alni* consisted of one to many irregularly shaped colonies of differing size and optical density. Because of these irregularities, differences between visual estimates of disease severity and actual values calculated with a planimeter and values calculated from video image analysis increased with the fraction of leaf surface diseased. However, while errors in measurement using visual assessment keys increased with increasing fractions of necrotic leaf surface, errors in estimates of necrotic leaf surfaces calculated from video image analysis were small and nearly independent of the fraction of necrotic leaf surface (Fig. 5). Similarly, the area of numerous small necrotic lesions caused by *A. solani* on the compound leaves of tomato calculated with the automated disease assessment device were highly correlated with the actual areas measured with a planimeter (Fig. 6). Even the area of numerous highly irregular necrotic lesions of *A. pteridium* on the bipinnately compound fronds of bracken fern computed by video image analysis closely matched the actual areas of necrotic tissue measured with a planimeter (Fig. 7). While the areas of necrotic lesions calculated by video image analysis were highly correlated with actual areas of necrotic lesions, the automated disease assessment device appeared to underestimate necrotic areas consistently by approximately 7.0% (Fig. 7). This was attributed to small darkly pigmented areas within certain necrotic lesions that were not correctly identified by our system as necrotic lesions on the basis of differential brightness. Because

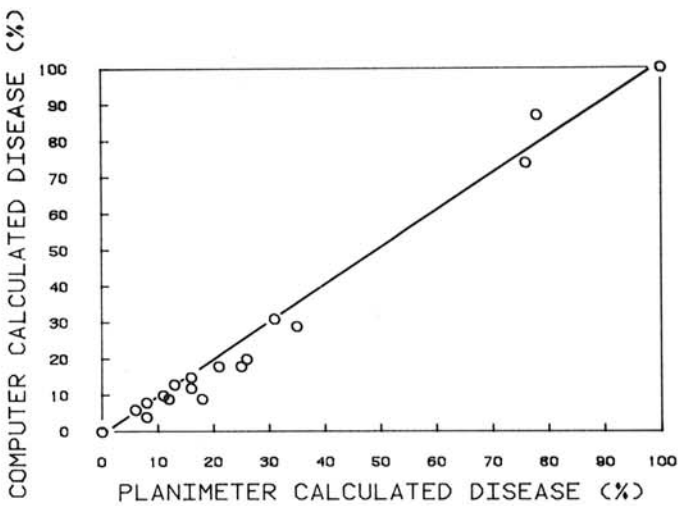

Fig. 6. Relationship between percent of necrotic tomato leaf area infected with *Alternaria solani* as determined from leaf tracings with a planimeter (abscissa) and as computed from video image analysis (ordinate). The line drawn represents the linear regression $y = 1.06x - 4.35$; coefficient of determination = 0.97 ($P < 0.001$).

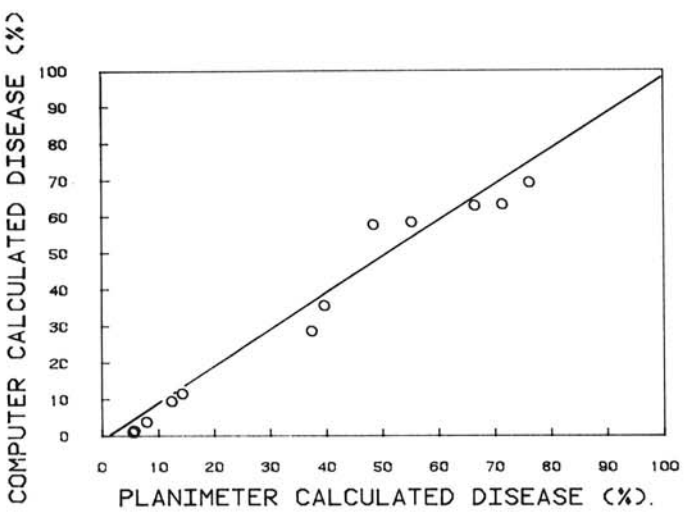

Fig. 5. Relationship between percent of sycamore leaf area covered with *Microsphaera alni* as determined from leaf tracings with a planimeter (abscissa) and as computed by video image analysis (ordinate). The line drawn represents the linear regression $y = 1.04x - 1.24$; coefficient of determination = 0.992 ($P < 0.001$).

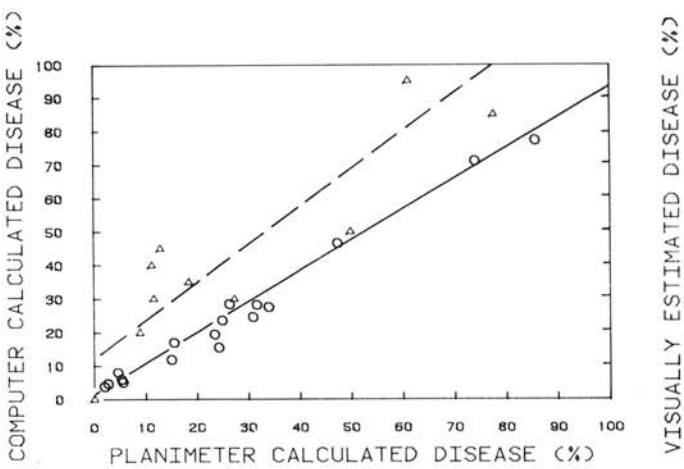

Fig. 7. Relationship between percent of necrotic bracken fern frond area infected with *Ascochyta pteridium* as determined from leaf tracings with a planimeter (abscissa) and as computed by video image analysis (ordinate) (o) or as estimated using visual assessment keys (Δ). The solid line drawn represents the linear regression $y = 0.914x - 0.17$; coefficient of determination = 0.976 ($P < 0.001$) for the relationship shown in circles.

errors in the computer estimates of necrotic frond area were repeatedly found to be linearly related to fraction of frond areas diseased, automated corrections could be made for the small portions of lesions that were not quantified by the system. The correction permitted estimates of necrotic frond area for which error was independent of the magnitude of the determination. Estimates of necrotic frond area using visual assessment keys were much less accurate than estimates made from video image analysis (Fig. 7). Whereas estimates of necrotic frond area from visual assessment keys approached those from video image analysis and the true areas at both low and very high disease severity, large errors were incurred at intermediate disease severity levels (Fig. 7).

The accuracy of disease severity estimates made by video image analysis was high and independent of the irregular shape of the leaf, the type of disease symptoms, and disease severity. With the exception of ascochyta leaf spot of bracken fern, regressions of disease severity by the automated disease assessment device on severity determined with a planimeter had slopes very close to 1.0 (Figs. 4–7). The 95% confidence limits for individual determinations averaged approximately within ±1.0% of the expected value obtained by regression on each of the four diseases that were examined.

DISCUSSION

The automated disease assessment device developed in this study utilizing computer-controlled digital video image analysis is a very rapid and highly accurate system for measuring severity of several foliar diseases. Measurement of foliar diseases by such a system should be quicker, more accurate, and less expensive than interpretation of false-color infrared photographs (6,13). The computer-contolled automated disease assessment device developed in this study was designed to be largely self-correcting for variations in system performance and the disease under investigation. Internal correctional algorithms utilized by the computer allowed repeatability with a 95% confidence interval of <1.0%. Similarly, the enhanced contrast of necrotic lesions under the red illumination used in this device allowed the system to be highly versatile. Difficulty in measurement of only very darkly pigmented lesions was encountered with the system. Additional improvements in system performance could be made by optical changes such as measurement of near-infrared radiation instead of far-red radiation or illumination with other wavelengths to allow measurement of very dark lesions. A similar system to that described, but utilizing a light-colored background to allow differentiation of darkly pigmented lesions, has also been developed.

The entire automated disease assessment device described in this study is relatively inexpensive (~$3,000). If an Apple II Plus microcomputer is already available, the system can be implemented for much less cost. Expensive image analysis devices have recently been utilized to measure discoloration and decay in trees (1) and certain foliar diseases (3,10). These devices utilized some aspects of video image analysis used in this study and accurately assessed diseases of their respective plants, but primarily were used to analyze photographs of plants (10). These systems are not as versatile as the system described here and may be prohibitively expensive for use in most laboratories.

The microcomputer-directed video image analysis system developed in this study was shown to be more accurate and reproducible in measuring foliar diseases than the visual assessment keys in current use (7). Whereas the system described above is a laboratory-based device, measurements of symptomatic leaves in the field should be possible by means of analysis of remotely recorded video images. Since the precision of estimates of foliar disease severity utilizing video image analysis will vary inversely with the dimensions of the plant sample analyzed, the more leaves or plants included in one observation to obtain a representative mean disease severity of a group of plants, the less accurate will be such determinations. Therefore, a more precise determination of mean disease severity may be obtained by random sampling of a larger number of individual leaves or plants for which a more precise estimate of disease severity can be made. The speed and accuracy of the automated disease assessment device would be ideal for such a purpose. Sample size then becomes critical. This in turn depends on the pattern of development of disease in the field. Similarly, symptomatic leaves of many foliar diseases are within the plant canopy and thus may not be detected by remote sensing. The automated disease assessment device described would be useful in determining disease severity on a large number of individual leaves to determine average disease severity of infected foliage. Because the results of measurements of foliar diseases by the automated device are automatically placed in the microcomputer, rapid statistical analysis of results is possible without further data manipulation as would be required of visual or other assessment methods. The uses of video image analysis in plant pathology research should be many and varied. Since both total and necrotic leaf area of intact plants can be monitored by video image analysis, this procedure should be useful for in situ measurements of plant growth as influenced by plant pathogens or the dynamics of lesion expansion of many foliar pathogens. Measurements of disease severity by digital video image analysis will be useful for testing and developing models of quantitative epidemiology and in studies of plant pathogens as biological control agents of undesirable host plants.

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