MEASURING MITE FEEDING DAMAGE ON AVOCADO LEAVES WITH AUTOMATED IMAGE ANALYSIS SOFTWARE

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One way of assessing the impact of pathogens or herbivores on host plants is to measure the area of leaf damaged. Common methods to estimate the area damaged include counting the number of squares with damage on a grid overlayed on the leaf surface (Simms 1993) or visually estimating leaf area damaged by comparison with pictures of leaves with known amounts of damage (Schaffer et al. 1986, Kogan & Turnipseed 1980). These techniques are simple to implement; however, they are not accurate. Injury in the form of either excised or necrotic tissue is often split into several small areas of various and irregular shapes. Accurate damage estimates require precise measurements; this may be achieved with automated image analysis techniques (McMillan & Schwartz 1993, Kokko et al. 1995, Schaffer et al. 1997). In this study we evaluated the performances of three techniques within a single image analysis software package to measure leaf damage caused by a phytophagous mite.

The persea mite, *Oligonychus perseae* Tuttle, Baker and Abbatiello (Acari: Tetranychidae), is a serious pest of avocados, *Persea americana* Miller (Lauraceae) in California. Mites live and feed inside silk nests on the underside of leaves (Aponte & McMurtry 1997). Feeding by mites induces necrosis of leaf tissue forming the nest's floor resulting in a pattern of small (ca 1-5 mm^2) brown spots located primarily along leaf veins. In order to study susceptibility of various avocado cultivars to O. *perseae*, we wanted to quantify mite feeding damage using an image analysis technique to count brown spots on leaves and calculate percentage of leaf area damaged. To determine the most accurate technique, we evaluated three measurement methods within the software package. The software used automatically marked and measured areas on a scanned leaf image. To determine that the software marked all damaged areas, we compared results obtained with these three methods with results obtained when damage was marked manually with the computer mouse on scanned images. Manual marking was considered the most accurate non automated method of measurement to which automated methods could be compared.

Mature leaves with *O. persea* feeding damage from the summer of 1997 were collected in February 1998 from an avocado orchard of mixed cultivars grown at South Coast Experiment Station, Irvine, CA. In the laboratory, 14 leaves were individually scanned (petiole removed) with a flat bed color scanner (UMAX® Astra 1200S; resolution 400 dpi and 25% size reduction) to obtain true color pictures which were saved as BMP format files.

Leaf damage was measured with SigmaScan™ Pro 4.02 (Jandel Corporation 1995) image analysis software. Macros were written to perform automated measurements of damage on leaves with each of the three methods described below:

(1) *Color-defined marking method (CD):* the program was set to mark all brown areas (pixels with hue values of 0-35 which was the color of necrotic tissue caused by mite feeding).

(2) *Filtered color-defined marking method (FC)*: the program was set to mark all brown areas (hue 0-35). Then, marked areas were filtered with a binary erosion filter to separate fused marked areas into distinct spots.

(3) *Shape-defined marking method (SD)*: the program was set to mark all brown areas (hue 0-35). Then, the shape factor (S) of marked objects was calculated with the following equation: $S = \frac{4\pi a}{p^2}$, where a = area and p = perimeter. Brown areas with S <

0.55 were ignored because preliminary measurements showed that S > 0.55 for necrotic spots caused by mite feeding.

For each method, the number of marked spots of areas larger than 1 mm^2 (smallest estimated size of necrotic spots due to mite feeding), was automatically counted, and the total area marked was measured to compute percentage of leaf area damaged. Total leaf area was measured as the sum of all colored pixels in the picture.

Of the three automated methods, the CD method gave the closest results to the manual method both for the number of necrotic spots and the percentage leaf area damaged (Fig. 1). Deviations of automated damage measurements from manual measurement were relatively constant across leaves with all methods (Fig. 2). Only percentage area damaged for leaves with very little mite feeding damage but extensive

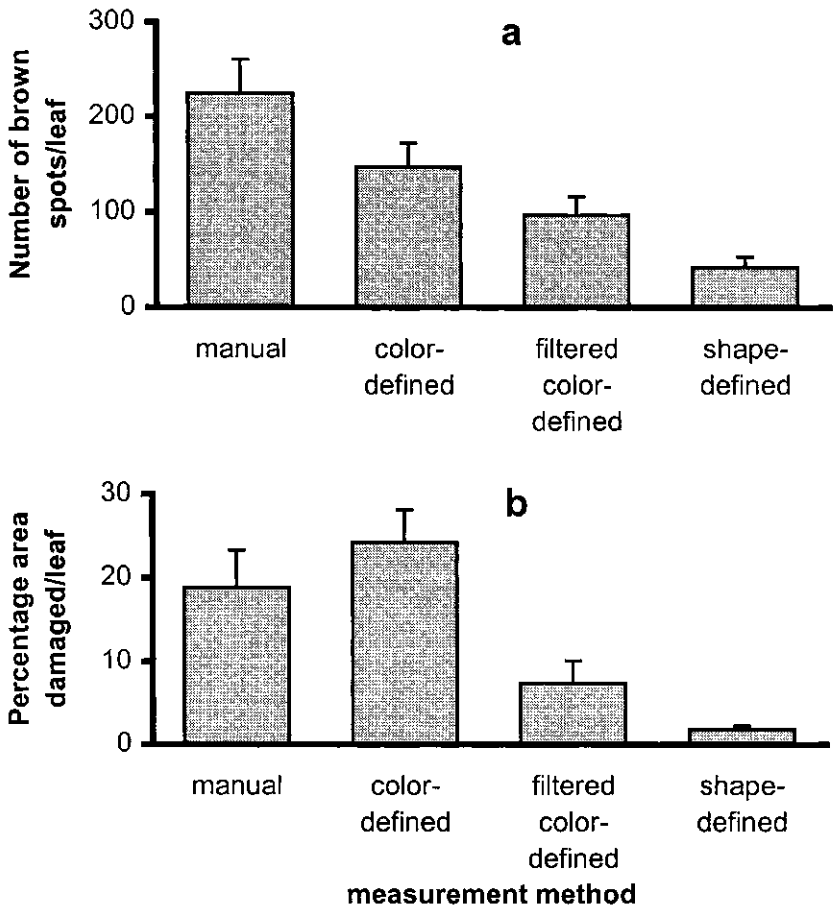

Fig. 1. (a) Mean number of necrotic spots (+ s.e.) and (b) mean percentage of leaf area damaged (+ s.e.) by *Oligonychus perseae* when measured manually or with three automated image analysis techniques (N =1 4).

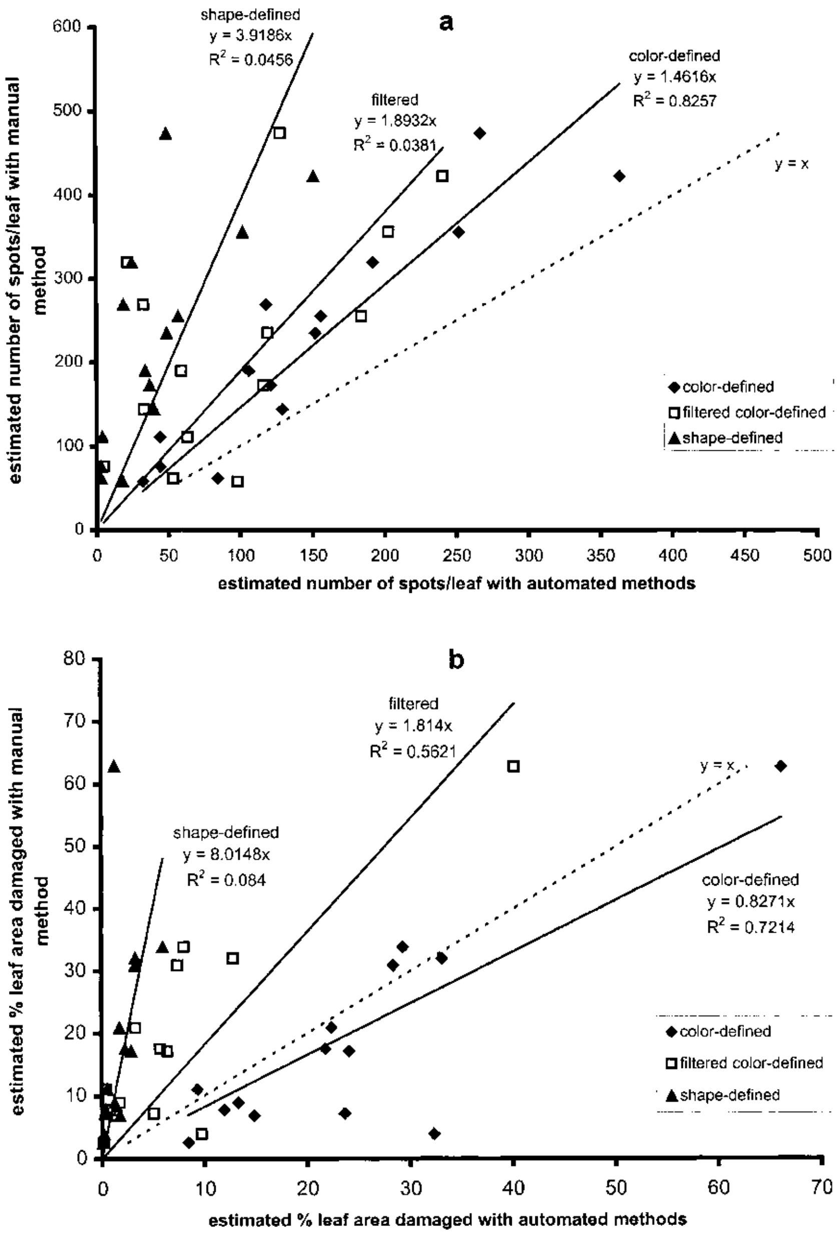

Fig. 2. Linear regression between estimates of damage caused by *Oligonychus perseae* on avocado leaves when measured with one of three automated techniques and manual measurements. (a) Number of necrotic spots per leaf and (b) percentage area of leaf damaged.

additional damage (e.g. tip burn) tended to be overestimated with CD marking. However, this problem can be readily overcome by masking the area of non-mite damage before automated measurements are made. With the manual marking method, 225 necrotic spots were counted per leaf on average (Fig. 1a). The CD, FC, and SD methods significantly underestimated the average number of spots by 35, 57, and 81%, respectively (Repeated-measures ANOVA, $F = 27.36$, $df = 3$, $p = 0.0001$). The average percentage of leaf area damaged was overestimated by 29% with the CD method compared to the manual method (Fig. 1b). However, percentage of leaf area damaged was significantly underestimated by 61% with the FC method and by 89% with the SD method (Repeated-measures ANOVA on Arcsine-transformed data, $F = 47.08$, $df = 3$, $p = 0.0001$).

Although CD marking underestimated numbers of spots (by merging spots together) and overestimated the percentage of leaf area damaged (by marking dark healthy leaf tissue), damage was estimated accurately with this method by applying correction factors of 0.68 for the number of spots, and 1.21 for the percentage area damaged to measured values.

SUMMARY

Of the methods tested within the automated image analysis software package, color-defined marking was the most accurate compared with manual measurements for measuring mite feeding damage on leaves. This technique will be very useful in studies involving numerous measurements of leaf damage because it (1) provides reliable damage estimates, (2) is fast (<2 min to scan and measure damage on a leaf), (3) provides a permanent record (pictures stored on disks), and (4) results do not vary among people because measurements are automated.

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