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SHORT COMMUNICATION

A photographic technique for tracking herbivory on individual leaves through time*

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Introduction

Accurate measurement of leaf area removed (LAR) by chewing insect herbivores is used to evaluate progress in insect control, plant breeding, and genetic screening and engineering programs, and to assess impacts of herbivores on ecosystem function (Bray, 1961; Schowalter *et al.*, 1986). End-of-season estimates of LAR may underestimate herbivory because of hole growth with leaf expansion (Reichle *et al.*, 1973) and consumption of entire leaves (Lowman, 1984). This report describes a method for following herbivory through time, yielding more accurate estimates and indicating the seasonal dynamics of herbivory.

Manual methods for estimating LAR of attached leaves in the field are labour-intensive, and require that this labour be done under field conditions. These non-destructive techniques (e.g. length on area regression, silhouette tracing (Milthorpe, 1956); manual integration using dot- or square-counting (Benjamin *et al.*, 1968; Negisi *et al.*, 1957)) are slow and tedious, operator dependent, and require considerable manipulation in the field. The effort required has precluded their widespread use.

Most automated techniqúes for the measurement of LAR involve removal of leaves from the plant. Video integration methods require less effort (Hargrove & Crossley 1988), but are too complex or bulky for field use. This is also true of most optical, mechanical, magnetic and photometric planimetry devices that have been described (Hargrove & O'Hop, 1988; Milthorpe, 1956; Negisi *et al.*, 1957; Pedigo *et al.*, 1970). Since few of these devices are portable, removal of foliage is necessary.

More importantly, mechanical damage to leaves may stimulate plants to increase concentrations of chemical feeding deterrents in other leaves remaining on the tree (Schultz & Baldwin, 1982; Haukioja & Niemela, 1977; Ryan, 1983). These facultative defences, induced by the action of herbivory, may also be induced by pruning foliage samples for analysis. Inducible defences may be widespread; one type has been found in members of twenty plant families. Destructive examination of LAR may affect subsequent consumption by insects.

The proof paper method

Non-destructive measurement of LAR is possible with a technique employing photographic printing-out paper or studio proof paper (Kodak Studio Proof Paper, 8×10 in., Cat. 143 3168). Photographic developer is incorporated directly into the emulsion of this paper, causing a visible darkening with exposure to light. Thus, prints require no wet chemical development process for viewing.

Proof paper is used commercially by portrait studios for the dissemination of proof portraits to potential customers. The developer continues to act with ambient exposure, and the image

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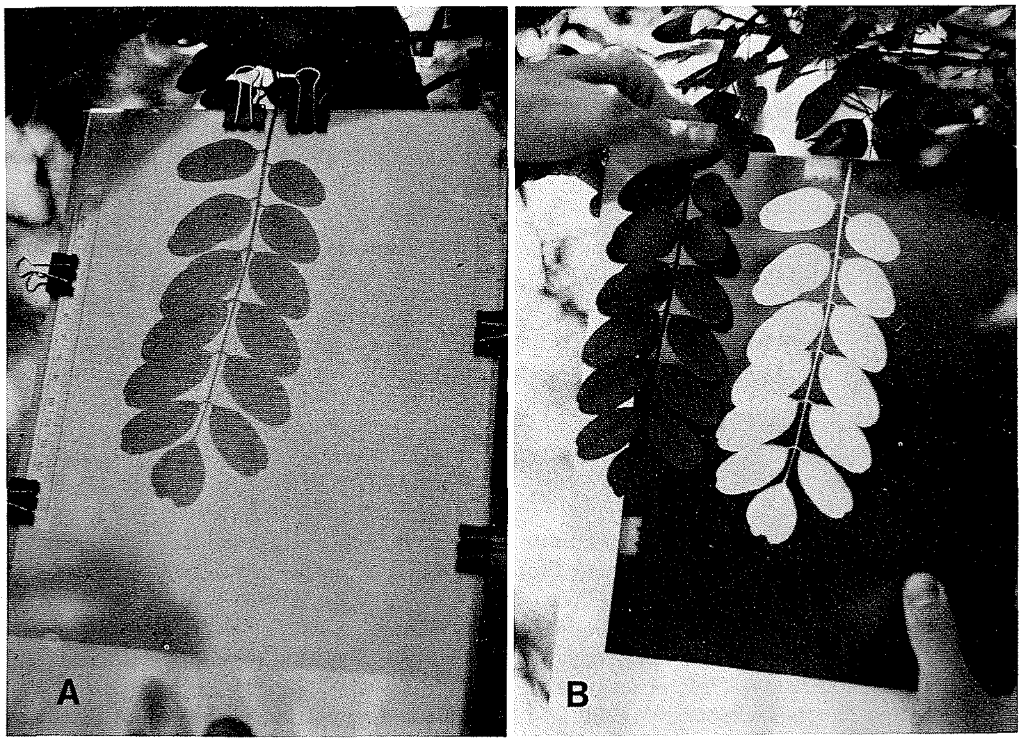

FIG. 1. (A) The leaf is gently pressed between two thin plexiglas sheets, the Kodak Studio Proof paper slipped in behind it, and the sheets clipped together along the edges. The emulsion of proof paper is slow enough that there is little darkening before the sandwich is assembled. (B) Complete exposure takes about 1.5 min in full direct sunlight. The entire darkening process is visible. The finished contact print is a full-size negative image of the leaf.

eventually becomes a uniform shade of magenta, presumably ensuring the customer's return to the studio for purchase of fixed portraits.

Studio proof paper is ideal for making real-size contact prints of living, attached leaves. The leaf is gently pressed between two thin plexiglas sheets and the photographic paper slipped in behind it. There is little darkening before the proof paper is in place and the plexiglas 'sandwich' is assembled. The 'sandwich' is clipped together with small binder clips along the edges (Fig. 1A).

Complete exposure takes about 1.5 min in full direct sunlight; cloudy skies increase exposure time. Early and late in the day, longer exposures are required. Exposure time may be up to 5 min on overcast days. The darkening process is visible, so no calculations are necessary for proper exposure. The finished print is a uniformly dark magenta colour.

The resultant print is a real-size duplicate image of the leaf (Fig. 1B). Holes are easily visible in the negative leaf image. Leaves of many species are sufficiently translucent that, with a long exposure, areas of overlap will show through.

Tracking herbivory through time

The proof paper method can be used serially through time to document incremental herbivory on individual leaves. Single compound leaves on each of twenty-four black locust (*Robinia pseudoacacia* L.; Fabaceae) trees on watershed 7 at the Coweeta Hydrologic Laboratory, 40 km south of Franklin, N.C., U.S.A., were imaged in June, July, August, September and October 1983. Since watershed 7 was in the seventh year of secondary regrowth following clearcutting, canopies were accessible from the ground using a 4 m stepladder. During the five mid-monthly imaging dates, 150 proof paper images were made.

Leaves were marked using sticky fluorescent labels. Five to seven tagged leaves were kept on each tree at each of the five intervals. If a leaf had dropped from the tree, a new one was selected to replace it. This rotating marking process provided an internal control for effects of imaging on leaf abscission and accrual of LAR. By lumping consumption and abscission data for the July–August and August–September intervals into two categories, those leaves which had been previously imaged and those for which this image was the first, the effects of marking and imaging could be evaluated. For neither of these intervals were previously-marked leaves significantly different from those which had not been previously photographed in terms of number of leaflets or degree of herbivory (two-sample t-test, $P<0.05$), suggesting that imaging and marking had no significant effect on abscission or LAR.

Because proof paper is slow and relatively insensitive to artificial lighting, unfixed prints can be viewed under subdued lighting for up to 2 weeks with little deterioration in image quality. A wet chemistry fixing process results in permanent glossy prints impervious to further change. Development is halted by 3 min in a 3% acetic acid stop bath, and up to 3 min (less for dark prints) in hardening fixer. Sodium hypochlorite clearing agent and a running-water rinse remove excess silver and developer. A print drier at a fast apron speed can be used to dry and gloss the images. A darkroom is not required; all steps can be done under artificial illumination.

After fixing prints, leaf outlines were reconstructed by extrapolating leaf margins, and each hole was identified with a unique number. The simple elliptical leaflets of black locust can be reconstructed with little error (Hargrove & Crossley, 1988). The size of leaves and holes and % LAR were measured using a Hewlett-Packard HP 9825A microcomputer with a magnetic digitizer board (Hargrove & O'Hop, 1988). The outline of each leaflet and each hole was traced using a gunsight-type magnetic cursor. The result is a printout of all holes by number, their size, and %LAR for each leaf.

When successive proof-paper images of the same leaf are sequentially examined, the herbivory history of each leaflet can be reconstructed. Individual leaflets and holes can be uniquely identified through the photographic series. Abscission of leaflets is documented, as well as addition and enlargement of holes. Centimetre scales at the edges of each image were used to test for paper stretching during wet chemical processing, but no significant changes in physical dimensions were found.

Results

A subset of these data, representing about 500 holes, was analysed with regard to hole 'behaviour' through time (Table 1). The five imaging dates define four intervals through which all damaged areas present could be classified as 'new initiations,' 'enlargements,' 'coalescences,' 'shrinkages,' or 'no change,' depending on their individual fates during that interval. Enlargements represent holes increasing in size by more than 5 mm^2. Two damaged

TABLE 1. 'Behaviour' of 520 damaged areas in compound leaves of *Robinia pseudoacacia* L. through 5 months of 1983. Holes present in intervals between images were classified as 'new initiations,' 'enlargements,' 'coalescences,' 'shrinkages,' or 'no change.' Coalescences scored as a distinct subset of enlargements. Most holes remain static through time. A greater percentage of holes were new initiations than enlargements of existing ones.

	June–July	July–Aug.	Aug.–Sept.	Sept.–Oct.	Total
Enlargements	34 (9%)	4 (1%)	19 (10%)	4 (3%)	61 (6%)
Coalescences	12 (3%)	0 (0%)	13 (7%)	0 (0%)	25 (2%)
Initiations	64 (17%)	78 (23%)	7 (4%)	3 (2%)	152 (14%)
Shrinkages	11 (3%)	2 (0%)	10 (5%)	4 (2%)	27 (3%)
No change	247 (67%)	258 (75%)	147 (75%)	152 (93%)	804 (75%)
'Active' holes	121 (33%)	84 (25%)	49 (25%)	11 (7%)	265 (25%)
Total	368 (71%)	342 (66%)	196 (38%)	163 (31%)	1069

areas enlarging and joining together were scored as a coalescence. To coalesce, holes must enlarge; coalescences were scored as a distinct subset of enlargements. Shrinkage was scored if hole size decreased by more than 2 mm^2. Holes present at the start and finish of the interval but not meeting the above criteria were scored as 'no change.'

Herbivore activity, reflected by the first three categories (Table 1), was greatest in the June–July interval and decreased with time, stressing the importance of early-season herbivory at Coweeta. New initiates predominated, but there were also moderate enlargements and shrinkages. The September–October interval showed very little herbivore activity. The greatest percentage of holes present showed no change.

New initiations during the July–August interval increased to nearly a quarter of all holes present, dwarfing total enlargements by comparison. A late-season increased in herbivore pressure is well-documented on watershed 7 at Coweeta (Schowalter *et al.*, 1981). There was no shrinkage during this interval.

In the August–September interval there was a shift as enlargements and coalescences surpassed new initiations. More holes were shrinking as nutrients were translocated out of leaves, and they dried and senesced in preparation for leaf drop. In September–October, total enlargements equalled new initiations, but both were waning in total proportion. Shrinkage increased in frequency. Percentages of total scores closely resembled proportions for the June–July interval.

Conclusions

Most holes showed no change in size, even during intervals of rapid leaf expansion. Enlargements could be caused by leaf expansion or herbivore feeding, but coalescences could not arise from hole growth due to leaf expansion. Leaf growth may have been responsible for enlargements during the first interval, but enlargements in August–September, well after leaf expansion, are probably herbivore-induced. Only 9% of holes enlarged during the first interval. Shrinkage in the last two intervals was likely caused by senescence and drying. End-of-season estimates of %LAR for black locust are not upwardly biased because of leaf growth, and may indeed be conservative.

Many herbivores chose to initiate new holes rather than enlarging existing ones. Even during the July–August interval, when new initiations represented nearly a quarter of all holes, there were few enlargements, and no coalescences. Most black locust herbivores are small and highly mobile (Hargrove, 1986); there was no advantage to resuming feeding on an existing edge over biting through anew.

Preference for new initations over enlargement of existing holes might indicate a localized inducement of feeding deterrent compounds in the immediate area surrounding an older hole, forcing herbivores to find a new, uninduced spot for the next feeding site (see Edwards & Wratten, 1983). Alternatively, local drying of mesophyll in the vicinity of fresh edges, accelerated by disruption of the waxy epidermis, may reduce palatability in the areas adjacent to an old hole. Leaf moisture has been positively correlated with palatability and nutrition to herbivores (Scriber, 1977, 1979). Preference for new initiations was especially strong in the first two intervals, but relaxed later in the season.

If the halo-inducement hypothesis is true, holes should be overdispersed in leaves (Edwards & Wratten, 1983). Spatial analysis of proof paper images could test this. The proof paper method could also determine if early abscission of leaflets is correlated with degree of insect damage, and whether propensity for herbivory is correlated with leaflet position on the rachis. A detailed record of leaf and hole allometry through time could be used to correct static single-point %LAR estimates for leaf and hole growth. The proof paper technique is a simple and powerful tool for the non-destructive tracking of herbivory through time.

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