# An Assessment of Techniques for Measuring Herbivory: Is Rainforest Defoliation More Intense Than We Thought?[1]

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## ABSTRACT

Amounts of leaf material grazed by insect herbivores in Australian rainforest canopies were measured over 3 years, and two methods of assessment were compared. Long-term observations of labelled leaves and shoots measured losses up to five times larger than estimates obtained by the more conventional technique of measuring missing leaf area on single occasions. Long-term observations gave higher but more accurate rates since they incorporated a temporal component to grazing activities and also accounted for leaves totally eaten. Annual leaf area losses of 26, 22, and 14.6 percent were measured in Australian cool temperate, warm temperate, and subtropical rainforests, respectively. Grazing in some plant communities may be higher than previously recorded from discrete measurements of missing-leaf area, resulting in an underestimation of the impact of herbivory.

Most studies of herbivory in forest communities have estimated grazing by harvesting discrete samples of leaves and estimating proportions of leaf area missing. This method usually indicates leaf area losses of from 3 to 10 percent and is similar in tropical forests (Odum and Ruiz-Reyes 1970, Benedict 1976), coral island shrubs (Lowman in press), European beech woods (Nielson 1978), and northern temperate hardwood forests (Bray 1961, 1964; Woodwell and Whittaker 1968, Reichle *et al.* 1973, and many others). This method—termed "discrete sampling" since leaves are selected, usually at one point in time, and measured for apparent leaf damage—provides a quick and easy means of estimating herbivory as reflected by partial defoliation of leaves. It may, however, underestimate the real herbivory losses of forest canopies, since it does not account for leaves totally eaten.

Two methods of discrete sampling commonly are used: (1) leaf samples removed from litter traps and measured for hole damage (*e.g.*, Odum and Ruiz-Reyes 1970), or (2) leaf samples picked from tree canopies (*e.g.*, Fox and Morrow 1983). Assuming that mature leaves are picked in method 2, then the two sampling techniques are very similar: both measure partial defoliation incurred during the lifespan of a leaf, but the samples include only uneaten or partially eaten leaves and do not account for totally eaten leaves.

A more comprehensive method of quantifying herbivory, long-term observations, was developed in this study to incorporate a temporal component into the sampling and also to monitor the number of totally grazed leaves. This technique entails repeated observations and measurements of permanently marked leaves, providing an accurate measure of defoliation and the temporal aspects of grazing activities.

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In view of the increasing interest in plant–insect relationships, the different methodologies for measuring herbivory are compared in this study for discrepancies that could arise. When similar long-term sampling techniques (albeit only over 3 months) were used in the neotropics (Coley 1983), grazing losses reported were higher (21%) than those in previous studies (7%; Odum and Ruiz-Reyes 1970). Similarly, grazing estimates of Australian eucalypts that accounted for leaves totally eaten (although estimated by petiole counts rather than actual observations) were higher than losses measured by discrete sampling (Journet 1981). Discrete samples of coral cay shrubs also revealed lower defoliation losses (2–3%; Lowman, in press) than long-term observations made previously (up to 30%; Heatwole *et al.* 1981). However, none of these examples featured direct comparisons between discrete leaf samples and long-term observations at the same time and site.

In this study, two major questions concerning herbivory were addressed: (1) What are the levels of defoliation in Australian rainforest canopies? (2) What are the discrepancies (if any) between discrete leaf sampling and long-term observations in assessing herbivory?

## STUDY AREAS

Field work was conducted in canopies of three rainforest formations in New South Wales, Australia, where rainforests are distributed discontinuously along the coast. Profile diagrams and detailed site descriptions are listed elsewhere (Lowman 1982a). The three formations, described according to Webb's (1959) classification, included the following sites. 1. Cool temperate rainforest or microphyll fern forest in New England National Park (30°30'S), a montane rainforest situated at 1200 m on the New England plateau and predominated by one species (*Nothofagus moorei*, Antarctic beech) in the canopy with 5–10 understory trees (including *Doryphora sassafras*). Three subsites, each comprising an isolated pocket of beech forest at least 1 ha in size and 2 km from one another, were sampled to examine spatial variability of herbivory rates within one species. 2. Warm temperate rainforest or simple notophyll vine forest located in Royal National Park (34°10'S), with a canopy composed of 10–15 major species (including *D. sassafras*, *Toona australis*, and *Ceratopetalum apetalum*). 3. Subtropical rainforest or complex notophyll vine forest located in Dorrigo National Park (region 1) (30°20'S) and Mt. Keira Reserve (region 2) (34°30'S), whose canopies have high species diversity (15–30 species including *Dendrocnide excelsa*, *D. sassafras*, and *T. australis*) and whose structural features were the most complex of the three formations examined.

All species that occupied a major portion (≥5%) of the canopy were sampled for herbivory. In addition, five species that dominated particular rainforest formations were selected for observations over 2 years: *C. apetalum*, a common canopy species in the warm temperate formation; *D. sassafras*, a canopy dominant in the subtropical and warm temperate formations, and an understory species in the cool temperate formation; *D. excelsa*, a canopy dominant in the subtropical rainforest; *N. moorei*, forming a monospecific canopy in the cool temperate rainforest; and *T. australis*, a canopy species in the subtropical and warm temperate formations. In addition to their importance as canopy species in the rainforests, these species represented a range of leaf morphology and phenology (including soft and hard leaves, glaucous and hairy, evergreen and deciduous, etc.) that may be important in terms of grazing activities.

## SAMPLING METHODS

Herbivory was measured using two methods, termed "discrete" and "long-term" sampling. The two methods were compared at both the species and the community levels: first, herbivory (expressed as percentage of leaf area loss) was measured for individual species and included several sites and light regimes; and second, overall losses were calculated for each of three rainforest communities by averaging the percentages obtained for individual species according to the proportion of canopy occupied within the rainforest formation (leaf area index of each species calculated from leaf litter fall weights; Lowman 1982a).

*Discrete sampling* involved the harvesting of at least 200 mature (> 1 year old) leaves of each species. For each species, different leaf populations were sampled in multiples of 30 leaves, each leaf population representing a canopy region of a specific light regime, height, individual, or site. All leaf populations together constituted the herbivory for one species. To obtain reasonable standard errors, the number of leaves sampled was greater in cases where grazing was highly variable between leaves in one sample. Most samples were collected between January and March 1980 and repeated during 1981 to compare annual variability. The month of collection was not considered a critical factor, however, as long as only mature leaves were sampled. Rainforest leaves are most heavily defoliated during their first three months (Lowman and Box 1983); after maturation, defoliation is not significantly different between months (Lowman 1982a). Senescing leaves were avoided, since decaying leaf tissue can result in formation of leaf holes.

Leaves were harvested, sealed in plastic bags, and refrigerated until measurements of leaf area were made. The actual leaf area (ALA) of one side of each leaf was measured with a Lambda portable area meter (Model 300). The holes in the leaf surface then were covered with heavy tape and the leaf was measured again to determine potential leaf area (PLA), which is the total leaf surface prior to grazing. Then,

$$\left(1.00 - \frac{\text{ALA}}{\text{PLA}}\right) \times 100 = \text{\% leaf area lost to herbivores}$$

and

$$\text{PLA} - \text{ALA} = \text{cm}^2 \text{ holes per leaf.}$$

This value represents a direct measure of the amount of mature canopy missing, although it may be proportionally larger than the actual increments consumed by the herbivores when the leaves were young (Reichle *et al.* 1973). The use of proportions was considered a more useful expression than actual square centimeters since it was comparable among samples, regardless of leaf size variability; also, proportions of leaf hole remain constant before and after leaf expansion (Lowman 1982a).

*Long-term observations* involved monitoring permanently marked branches over 2 years (1979–1981) to record leaves completely defoliated. Complete defoliation cannot be accounted for without repeated observations of marked leaves. For each species, at least three branches were marked at different heights and light regimes for three individual trees in at least two sites. Three branches were marked at every 10-m interval (or as near as possible) up canopy trees including both sun and shade canopy regions. Ropes and technical climbing gear were used to gain access to the upper canopy (Perry 1978, Lowman 1982a). Leaves were numbered sequentially from the base upwards on branches that usually consisted of 8–15 leaves. Numbers were marked on the adaxial leaf surfaces with waterproof Pentel pens. The ink usually lasted at least one year before fading, at which time the numbers were remarked. New leaves were marked as they emerged. Monthly observations were made, and each leaf was measured for changes in leaf area missing as well as other aspects of mortality and growth.

To measure monthly increments of grazing without harvesting the leaves, a leaf tracing was made and the hole portions were calculated in the laboratory using the area meter (Lambda Model 300). Complete defoliation was distinguished from natural senescence by observation: older leaves that remained intact for several years and suddenly disappeared were recorded as having senesced; young leaves in which holes increasingly appeared over several months during times of herbivore activity (usually an obvious progression from 0–25% to 60–70% to 100%) were considered eaten.

The data on leaf longevity and monthly herbivory losses were collectively analyzed. Detailed temporal and spatial differences in defoliation of tree canopies will be reported elsewhere (Lowman, in prep.). However, average annual leaf area losses were compiled for each species from the long-term observations, and these data were compared to the discrete sampling.

## RESULTS

A comparison of the methods showed that results of discrete sampling were lower than those of long-term observation for each species (Table 1), indicating that herbivores consume some leaves entirely in rainforest canopies, rather than only partially grazing each leaf. The discrepancy between methods ranged from negligible difference (*T. australis*, where the long-term/discrete ratio was 1.1) to a highly significant ratio of 5.0 for sun leaves of *C. apetalum*, in which many leaves were entirely eaten. Overall, the long-term observations revealed a discrepancy approximately 2.5 times greater than the discrete sampling.

Within the canopies of individual species, variability in herbivory existed with respect to light (shade leaves were grazed more heavily than sun leaves, *e.g.*, *C. apetalum*: $F_{1,198} = 28.31$, $P < 0.001$) and with respect to sites, both on a small scale (*e.g.*, *N. moorei* among three subsites: $F_{2,117} = 3.03$, $P < 0.01$) and on a larger geographic level, although not significantly (*e.g.*, *D. sassafras* among three rainforest formations: $F_{2,117} = 0.55$, n.s.). Despite this intraspecific variability, the comparisons between sampling methods consistently showed a more conservative rate with discrete sampling as compared to long-term observations.

To estimate herbivory for the entire rainforest community (Table 2), the herbivory losses of many species and canopy regions were averaged using proportions weighted according to a species' abundance within a canopy (Lowman 1982a). The long-term calculations were two to three times greater than the discrete sampling. The cool temperate rainforest, with its monospecific canopy of *N. moorei*, had highest levels of grazing; the most diverse canopy, subtropical, had the lowest herbivory loss; and the warm temperate was intermediate both in species diversity of its canopy and in grazing.

Litter fall and leaf longevity studies were conducted in Australian rainforests simultaneously with grazing measurements and are reported in Lowman (1982a). The annual leaf fall data are summarized here, however, and compared with annual grazing (Table 3) to quantify the biomass turnover of these rainforest canopies along the two major pathways of primary consumers and decomposers. The total potential canopy in an evergreen rainforest where most leaves live 2 years (Lowman 1982a) comprises twice the annual leaf fall plus the leaf material grazed by herbivores. With 2-year leaf longevity, the proportion of annual leaf fall is only half that which occurs in a northern deciduous forest, whereas grazing damage is retained for twice the duration of an annually deciduous canopy. In the cool temperate rainforest, for example, the annual leaf biomass routed through to primary consumers (27%) is nearly equivalent to turnover through the decay pathway (36.5%). If grazing losses are as high in other plant communities, the effect of herbivores on plant primary production may be more extensive than previously assumed.

TABLE 1. *Differences between two sampling methods for measuring herbivory rates in Australian rain forest trees (and between populations of leaves within these canopies) (N = 200 leaves unless otherwise stated.)*[a]

| Leaf population | Sampling method for measuring herbivory rates (% leaf area consumed/year) Long-term | (SE) | Discrete | (SE) | Discrepancy ratio (L/D) | Sign.[b] |
|---|---|---|---|---|---|---|
| ***Toona australis*** | | | | | | |
| Subtropical | | | | | | |
| Region 1 | 6.3 | (0.4) | 5.7 | (0.3) | 1.1 | n.s. |
| Region 2 | 3.3 | (0.2) | 2.9 | (0.2) | 1.1 | n.s. |
| $\bar{x}$ | 4.9 | | | | 1.1 | |
| ***Dendrocnide excelsa*** | | | | | | |
| Subtropical | | | | | | |
| Region 1 | 32.5 | (2.6) | 16.5 | (1.0) | 2.0 | ** |
| Region 2 | 15.5 | (1.4) | 8.0 | (0.9) | 1.7 | * |
| $\bar{x}$ | 14.0 | | 12.3 | | 1.9 | |
| ***Ceratopetalum apetalum*** | | | | | | |
| Warm temperate | | | | | | |
| Sun leaves | 16.9 | (2.0) | 3.4 | (0.5) | 5.0 | ** |
| Shade leaves | 35.3 | (2.5) | 9.4 | (1.1) | 3.8 | ** |
| $\bar{x}$ | 26.1 | | 6.4 | | 4.4 | |
| ***Nothofagus moorei*** | | | | | | |
| Cool temperate | | | | | | |
| Subsite 1 ($N$ = 500) | 22.5 | (2.1) | 11.7 | (1.0) | 1.9 | * |
| Subsite 2 ($N$ = 500) | 40.5 | (1.8) | 15.0 | (0.9) | 2.7 | ** |
| Subsite 3 ($N$ = 150) | 30.3 | (3.0) | 13.6 | (1.4) | 2.2 | ** |
| $\bar{x}$ | 31.1 | | 13.5 | | 2.3 | |
| ***Doryphora sassafras*** | | | | | | |
| Cool temperate | 13.6 | (1.8) | 12.0 | (1.2) | 1.1 | n.s. |
| Subtropical | | | | | | |
| Sun leaves | 13.4 | (2.0) | 4.4 | (0.7) | 3.0 | ** |
| Shade leaves | 16.3 | (2.2) | 6.4 | (0.7) | 2.5 | ** |
| $\bar{x}$ | 14.9 | | 5.4 | | | |
| Warm temperate | | | | | | |
| Sun leaves | 17.6 | (2.2) | 4.5 | (0.9) | 3.9 | ** |
| Shade leaves | 27.8 | (2.6) | 7.6 | (0.9) | 3.7 | ** |
| $\bar{x}$ | 22.7 | | 6.1 | | | |
| $\bar{x}$ | | | | | 2.6 | |
| Overall mean ratio long-term/discrete sampling difference | | | | | 2.5 | |

[a] Populations of leaves are defined as groups of leaves with significantly different morphological features (*e.g.*, sun and shade leaves). Statistical data (ANOVA) for determination of leaf populations are listed in Lowman (1982a), and statistical differences in sun and shade leaf herbivory are reported in Lowman and Box (1983).
[b] Significance levels (students $t$-test) between long-term and discrete samples: n.s., not significant; * $P < 0.01$; ** $P < 0.001$.

TABLE 2. *Differences in herbivory rates for three Australian rainforest communities as reflected by two sampling methods.*

| Rainforest | Sampling methods for herbivory estimates[a] (% leaf area consumed/year) Long-term | Discrete | Discrepancy ratio (L/D) | Sign.[b] |
|---|---|---|---|---|
| Cool temperate | 26.0 | 12.7 | 2.0 | ** |
| Warm temperate | 22.0 | 6.2 | 3.5 | ** |
| Subtropical | 14.6 | 8.3 | 1.8 | * |
| $\bar{x}$ | 20.9 | 9.1 | 2.3 | |

[a] Each percentage was compiled from average leaf area losses for all major species in each habitat, with species weighed in the average according to its leaf area index. LAI was calculated from leaf litter fall amounts (Lowman 1982a).
[b] Significance levels (students $t$-test): * $P < 0.01$; ** $P < 0.001$.

TABLE 3. *Turnover of canopy material in three Australian rainforests (expressed as $t\ ha^{-1}yr^{-1}$).*

| Forest type | Potential canopy | Annual turnover of canopy[a] Herbivory | Decay |
|---|---|---|---|
| Cool temperate | 9.69 | 2.63 (27%) | 3.53 (36.5%) |
| Warm temperate | 11.41 | 2.50 (22%) | 4.05 (35%) |
| Subtropical | 12.26 | 1.76 (14%) | 5.59 (46%) |
| $\bar{x}$ | 11.2 | 2.30 (21%) | 4.39 (39%) |

[a] Numbers in parentheses refer to proportions of potential canopy.

## DISCUSSION

The removal of leaf material by primary consumers at proportions of up to 40 percent for some Australian rainforest trees is surprisingly high and suggests the need to reexamine the role of herbivores in turnover of plant biomass. Decay usually is regarded as a larger, more important pathway of leaf material turnover than grazing by primary consumers (Bray 1964, Bray and Gorham 1964, Wiegert 1970, Jordan 1971). However, in an evergreen canopy (where most leaves live more than one year), insect damage to young leaves remains incorporated into the canopy for twice the duration of annually deciduous leaves. Grazing losses of 3–10 percent, often cited in the literature from discrete measurements, may appear insignificant in terms of overall community biomass (Hairston *et al.* 1960). Herbivory may be more significant, however, if these figures represent two- or threefold underestimates, as in Australian rainforest.

Two possible explanations can be posed for these higher levels of herbivory—one relating to the isolation of Australian flora and fauna and subsequent differences in trophic levels, and the other simply an artifact of the sampling methodology. Recent studies in Australian plant communities have shown consistently high defoliation levels: eucalypt woodlands (Journet 1981), plantations (Carne 1966), uplands (Fox and Morrow 1983), and pastureland trees (Lowman, unpubl.); coral cay vegetation (Heatwole *et al.* 1981); and rainforests (Lowman 1982a, Lowman and Box 1983). Unusually large numbers of Coleoptera have been observed as defoliators in all Australian systems (Morrow 1977, Heatwole, pers. comm.; Lowman 1982b); whether the relative proportions of insect herbivores and insect predators in Australia are comparable to other continents remains unknown. Comparative intercontinental studies of insect and bird population dynamics may explain these apparent differences.

Assuming that trophic levels in Australian forests are similar to those of other continents, the high levels of herbivory may simply be an artifact of sampling. Long-term observations represent a more comprehensive method of sampling, which will inevitably yield higher results for plants in which some leaves are eaten entirely (an event unaccounted for in discrete sampling). This study shows consistently higher levels of defoliation as measured by long-term observations than by discrete sampling, corresponding to results elsewhere in the literature. Further comparisons of evergreen forest herbivory levels on another continent may indicate whether these discrepancies are a result of sampling or phenomena related to Australian plant–insect relationships.

It is possible that proportionally more leaves are entirely eaten in Australian rainforests than in other plant communities, thereby causing greater differences between sampling techniques than in other plant communities. It is also possible that the populations of herbivorous insects are higher in Australian rainforest than on other continents. Nonetheless, the apparent discrepancy obtained from the two sampling methods is worthy of consideration when assessing past studies of herbivory, since discrete sampling may have underestimated actual primary consumption.

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