The Blyolopsi 97(4), 1994, pp. 396-401


# Using Epiphyte Litter to Estimate Epiphyte Biomass

BRUCE McCUNE
Department of Botany and Plant Pathology, Cordley 2082, Oregon State University, Corvallis, OR 97331-2902

Abstract. *To estimate epiphyte biomass in tall forests, the only viable alternative to tree climbing methods is to sample litterfall. To evaluate the potential for using epiphyte litter to estimate epiphyte biomass, epiphyte litter and in situ epiphyte biomass were estimated independently in each of three* Pseudotsuga–Tsuga heterophylla *stands in the Cascade Range of Oregon and Washington, U.S.A. Litter was collected in late summers of two years in 20 2-m-radius plots. Groups of trees were felled and sampled for epiphytes. Samples were sorted into four groups: cyanolichens, alectorioid lichens, other lichens, and bryophytes. For lichens, biomass of litter (L) was strongly related to epiphyte biomass (B) ($r^2 = 0.87$, $n = 18$), in about a 100:1 ratio (fit as $\sqrt{L} = 0.1 \times \sqrt{B}$, yielding $B = 100L$). Year-to-year variation within stands was smaller than the differences among stands. Ten 2-m-radius plots are recommended for stands with simple structure, and 15 plots for older stands with more complex structure. The method is not recommended for bryophytes because bryophyte litterfall is highly aggregated. The method is recommended for large-scale surveys of lichens and other studies where large differences in epiphyte biomass are expected.*

Despite a growing awareness of the roles of epiphytes in montane and boreal forests (biomonitors of air quality, nitrogen fixation, and food for wildlife, e.g., caribou, deer, and small mammals) we know little of how epiphytes respond to and recover from disturbances (different kinds of cutting, wildfire). The most important barrier has been our inability to efficiently measure epiphyte biomass, given the inaccessibility of the organisms and their complex, three-dimensional substrate (Perry 1984). The problem is exacerbated for large trees in the Pacific Northwest. The brute force approach of removing and weighing epiphytes from a thorough subsample obtained through tree climbing is extremely time consuming. Concerted efforts as part of the International Biological Program in the early 1970's resulted in biomass estimates on only a few trees (Pike et al. 1972, 1977). Progress in this field has been hampered by the sampling problem.

An alternative method for estimating epiphyte biomass is to sample litterfall of epiphytes. It is obvious that forests with abundant epiphytes will have more epiphyte litterfall than forests with few or no epiphytes. But to make this relationship useful, the strength of the relationship must be demonstrated. If reasonably accurate, the method would have immediate applications to ecological studies; for example, biomonitoring studies of air pollutants, determining effects of various forest management practices on lichen forage for deer and mountain caribou, and studying the linkages between epiphytes, small mammals, and owls.

A study of processes in forest gaps (T. A. Spies, J. F. Franklin & C. Vogt, unpubl.) provided an unusual opportunity to accomplish this goal. Small groups of trees were felled in fall of 1990 in epiphyte-rich forests of various ages in the Cascade Range. We sampled epiphyte litter in the intact forest and compared that to estimates of in situ biomass based on sampling the felled trees. Biomass values and vertical gradients in biomass and species composition were reported in McCune (1993). The present paper describes the relationship between litter and in situ epiphytes.

## STUDY AREA

Three forests of different ages were sampled in the Cascade Range, Oregon and Washington. The old-growth forest (age 400+ years, elevation 915 m, 44°16'N, 122°9'W) was in the H. J. Andrews Experimental Forest, east of Eugene, Oregon. The younger stands (aged 95 and 145 years, elevations 550 and 500 m, 45°49'N, 121°53'W) were in Wind River Experimental Forest in southern Washington. The dominant species in all three stands was *Pseudotsugu menziesii* (Mirbel) Franco. *Tsugu heterophylla* (Raf.) Sarg., and to a lesser extent, *Thuja plicata* Donn., were the predominant understory trees. In the old stand, the *Pseudotsuga* trees often had broken tops and many of the *Thuja* and *Tsugu* were codominant in the canopy. The species list of epiphytes in Pike et al. (1975) is representative of the species occumng in these three stands. Although present nearby, nonparasitic vascular epiphytes (e g, *Polypodium)* were not present in these stands.

At the Oregon site, average annual temperature is 9.5"C, with January and July means of 2°C and 22"C, respectively (Waring et al. 1978). Average annual precipitation is 240 cm with 70% of that from November through March. At the Washington site, average annual temperature is 8.8"C, with January and July means of 0°C and 18°C, respectively

0007-2745/94/396401$0.75/0

(unpubl. climatological summary, Wind River Experimental Forest, 1911-1965). Average annual precipitation is 250 cm.

## METHODS

The objectives required independent estimates of standing crop of epiphyte litter and standing crop of epiphytes. These two sets of numbers are then compared by regression to assess the strength and shape of the relationship between litter and in-situ epiphytes. The regression equation is solved with in-situ biomass as the independent variable, because it presumably has less measurement error than litter estimates.

### EPIPHYTE LITTER

Epiphyte litter was sampled in late summer to avoid the large and variable pulses of litterfall that have been recorded in winter months for some forests (Esseen 1985; Stevenson & Rochelle 1984). This method is not intended to be used to estimate total annual litterfall. A single summertime sample cannot be used directly for this because lichen litter decomposes rapidly in these forests (McCune & Daly 1994), and most of the litter falls in the winter. Rather the goal was to estimate in-situ epiphyte biomass. Although one could also meet this goal with year-round collections from litter traps, the additional effort is not warranted if the method is to be used for surveying epiphyte biomass in a large number of stands.

To assess the year-to-year variation in late-summer standing crop of epiphyte litter, litter was gathered in two years: just before the tree cutting and two years later in the uncut areas of the same stands.

Epiphyte litter was gathered from 20 2-m-radius circular plots at randomized intervals averaging 20 m apart along transects through each stand. It was fast and effective to pace to a plot center from the previous plot, insert a chaining pin with a 2-m cord tied on it, then insert wire flags to mark four radii. The cord is used to check plot boundaries whenever it is uncertain whether a piece of litter is in or out.

Epiphyte litter was collected in no. 2 paper bags, air dried, then stored at room temperature until lab processing. In the lab, samples were dried at 60°C for 24 hr., then weighed to the nearest milligram. Litterfall from each plot was processed separately. Results were expressed in kg/ha.

To reduce the extremely labor-intensive task of sorting epiphytes by species, they were instead sorted into four functional groups: cyanolichens, alectorioid lichens, other lichens, and bryophytes. "Cyanolichens" includes all macrolichens with cyanobacteria as a primary or secondary photobiont (mainly *Lobaria oregana* with smaller amounts of other *Lobaria* species, *Nephroma, Pseudocyphellaria,* and *Peltigera).* "Alectorioid" lichens includes all pendulous species in the genera *Alectoria, Bryoria,* and *Usnea.* "Other lichens" includes all remaining macrolichens, mainly *Platisrnatia* and *Hypogymnia* species. Tufted *Usnea* species were included here, but because it is difficult to distinguish some small pendulous thalli from tufted species, I recommend combining the *Usnea* species into one functional group or the other, depending on the dominant species in the study area. Nomenclature of lichens follows Egan (1987) while that of mosses and liverworts follows Anderson et al. (1990) and Stotler and Crandall-Stotler (1977), respectively.

Preliminary sorting was done in the field, using a separate bag for each functional group for each plot. The following rules were used to standardize the method across plots:

1) Fragments less than 2 cm long need not be collected;
2) If the epiphyte litter has reestablished it is not collected. In most cases this is easily determined by observing thallus condition, orientation, and attachment to the substrate;
3) If the litter is attached to a fallen branch it is picked up unless the branch is attached to other branches with a diameter at its base of more than 10 cm. Thus litter attached to large fallen trees or branches is not collected, regardless of the location of the tree or branch relative to the plot;
4) Litter that is hung up in the understory at a height > 2 m above the ground is not collected;
5) Fragments are quickly cleaned as they are bagged. A final cleaning is done in the lab;
6) If the litter is largely incorporated into the forest floor (attached by fungal hyphae and partly buried by other litter), it is not collected.

Biomass values for individual species were determined by partitioning the measured biomass in each sample bag according to estimated proportions of different species. Each sampling bag was emptied onto a white enamel tray. The contents were teased apart and roughly sorted by species. Identifications of lichens were aided with spot tests and dissecting microscope, when necessary. Each species was then assigned a percentage of the biomass in that bag, by visual estimation. A complete sorting and weighing by species was prohibitively time consuming and tedious. The resulting information was then combined into a table of biomass for each species in each plot.

### EPIPHYTE BIOMASS SAMPLING

Epiphyte biomass on branches and trunks was estimated for individual felled trees, then extrapolated to the whole stands by regression techniques. The methods for direct sampling of the epiphytes and aggregation to the whole-tree and stand levels were described in McCune (1993). Lab processing was identical to that of the litter samples, except that proportions by species were not estimated.

To summarize, separate estimates for each functional group were made for branches and the main trunk on each tree. Biomass on trunks was estimated by stripping epiphytes from opposing 0.5 x 0.2 m quadrats at 4-6 m intervals up the trunks, or using 0.5 m cylindrical quadrats where trunk diameters were less than 20 cm. The appropriate multiplier was then used to adjust biomass to a g/m basis. These values were then numerically integrated along the length of the trunk to estimate the total biomass on the trunk.

Branch density (branches/m) and epiphytes on branches were sampled at intervals along the trunks. Small branches were stripped completely, while branches > 1.5 m long were subsampled by stripping epiphytes within four evenly spaced 0.5 m concentric bands (sub-subsamples), using a measuring tape nailed to the base of the branch. Biomass on each subsampled branch was estimated by numerical integration along the branch. Individual branch estimates were then aggregated to the whole tree by first multiplying branch density at each branch sample point by the corresponding single-branch epiphyte biomass estimate. The resultant branch epiphyte biomass values were then numerically integrated along the whole trunk.

### AGGREGATING FROM TREE-LEVEL TO STAND-LEVEL

Stand-level biomass of each functional group of epiphytes was calculated by using stand-specific regression equations relating epiphyte biomass to tree dbh (McCune 1993). The regression equations were then applied to extensive tree dbh data for the stand (M. Easter & T. Spies, unpubl.) to estimate epiphyte biomass on trees not included in the direct sample. Epiphyte estimates for individual trees were then summed to provide stand-level biomass estimates (McCune 1993). These estimates are probably somewhat low because of loss of epiphytes as the trees fall. This bias was partly compensated for by selection of branch and trunk samples that appeared to be least disturbed.

## RESULTS AND DISCUSSION

The biomass of epiphyte litter was more strongly related to epiphyte biomass in the canopy for lichens than for bryophytes (Fig. 1). *A* regression line was fit to the 18 points representing lichens. Each data point represents the biomass of one of three functional groups (alectorioid, cyanolichens, and "other" lichens) in one of three stands of varied ages at one sampling date (3 groups x 3 stands x 2 dates = 18 points). A square-root transformation was necessary to eliminate heteroscedasticity. An abundance of data might prove the relationship to be nonlinear, but this approach is not justified here, considering the sample size and the degree of scatter. Regression was not used for bryophytes because of the small sample size (n = 6) and the absence of a clear linear pattern.

The biomass of lichen epiphyte litter (L) was closely related to the biomass of epiphytes in the trees (B; $r^2 = 0.87$). It does not appear that the three functional groups of lichens differ greatly in their relationship between litter and canopy biomass. The small sample size, however, does not allow detection of subtle differences among functional groups in that relationship.

If the regression line is forced through the origin, so that there is zero epiphytic litter when there are no epiphytes in the trees, the resulting equation is: $\sqrt{L} = 0.1(\sqrt{B})$ with $r^2 = 0.87$ (both variables in kg/ha). It is fortuitous that the slope of this relationship is about 0.1, because back-transforming results in about a 100:1 ratio between lichen epiphytes and litter (Fig. 1). The actual strength of the relationship is likely to be at least slightly better than that reported here, because our estimates for canopy biomass from the direct measurement also have inherent error (McCune 1993).

Bryophytes in old forests often detach as large heavy mats composed of both bryophytes and a largely organic "soil," rather than as small fragments. This contributes to a sampling problem, and perhaps to the suggestion of nonlinearity in Figure 1, because the processes producing bryophyte litter differ between old forests and young forests. Furthermore, distinguishing between litter and forest floor inhabitants is more problematic for bryophytes than lichens. In some cases the large fallen polsters of bryophytes (e.g., *Antitrichia)* continue to thrive on the forest floor. Wisps of *Isothecium* appear to commonly reestablish on logs, shrubs, and low branches. In contrast, lichens falling from the canopy are likely to be falling into unfavorable habitat where they soon perish (McCune 1993; McCune & Daly 1994).

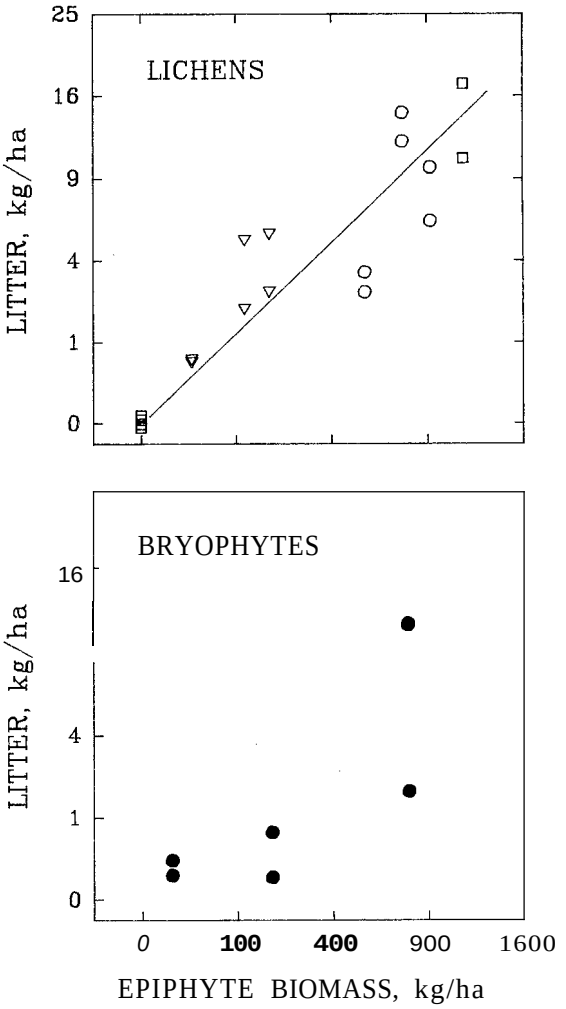


FIGURE 1. Relationship between biomass of epiphyte litter and biomass of epiphytes in trees. (Upper, lichens; lower, bryophytes). Each point represents one of four functional groups (solid circles = bryophytes, open circles = cyanolichens, triangles = alectorioid lichens, squares = other lichens) in one of three stands of different ages in one of two years. Litter samples were collected in late summer in *Pseudotsuga–Tsuga heterophylla* forests in western Oregon and Washington. The solid line is the regression line for lichens ($r^2 = 0.87$). The axes are plotted on square-root scales.

TABLE 1. Estimated biomass (B, kg/ha) and the standard deviation (S.D.) of that estimate (untransformed data) for canopy epiphytes in three stands, aged 95, 145, and 400+ years, based on litter sampling in the Cascade Range, Oregon and Washington, U.S.A.

| | 95 Years | | 145 Years | | 400+ Years | |
|---|---|---|---|---|---|---|
| Species | B | S.D. | B | S.D. | B | S.D. |
| Cyanolichens | | | | | | |
| *Lobaria hallii* | | | | | < 1 | < 1 |
| *L. oregana* | | | | | 998 | 994 |
| *L. pulmonaria* | | | | | 42 | 86 |
| *L. scrobiculata* | | | | | 1 | 2 |
| *Nephroma bellum* | | | | | <,1 | 1 |
| *N. helveticum* | | | | | 1 | 3 |
| *N. resupinatum* | | | | | < 1 | 1 |
| *Pseudocyphellaria anomala* | | | | | 3 | 6 |
| *P. anthraspis* | | | | | 5 | 10 |
| Alectorioid lichens | | | | | | |
| *Alectoria sarmentosa* | 7 | 21 | 306 | 738 | 93 | 97 |
| *A. vancouverensis* | 5 | 14 | 7 | 26 | | |
| *Bryoria capillaris* | < 1 | 1 | | | | |
| *B. friabilis* | 6 | 9 | 18 | 39 | 6 | 16 |
| *B. oregana* | < 1 | < 1 | 1 | 2 | < 1 | 1 |
| *B. pseudofuscescens* | 2 | 6 | 2 | 6 | | |
| *Usnea plicata* group | 20 | 36 | 36 | 57 | 16 | 50 |
| *U. scabrata* | 15 | 47 | | | 137 | 282 |
| Other lichens | | | | | | |
| *Cetraria chlorophylla* | 1 | 2 | 2 | 3 | | |
| *C. orbata* | 5 | 8 | 4 | 5 | < 1 | 2 |
| *C. pallidula* | | | | | < 1 | 1 |
| *C. platyphylla* | | | 18 | 24 | | |
| *Cladonia fimbriata* | | | | | < 1 | < 1 |
| *C. merochlorophaea* | | | | | < 1 | < 1 |
| *C. ochrochlora* | | | | | < 1 | 1 |
| *C. subsquamosa* | | | | | < 1 | 1 |
| *Esslingeriana idahoensis* | 7 | 10 | 14 | 13 | 2 | 4 |
| *Evernia prunastri* | 2 | 3 | < 1 | 1 | | |
| *Hypogymnia apinnata* | 1 | 2 | | | 1 | 5 |
| *H. enteromorpha* | 5 | 7 | 126 | 117 | 45 | 63 |
| *H. imshaugii* | 84 | 61 | 124 | 86 | 42 | 114 |
| *H. inactiva* | 337 | 269 | 239 | 138 | 69 | 147 |
| *H. metaphysodes* | | | 3 | 6 | < 1 | 2 |
| *H. occidentalis* | < 1 | < 1 | 1 | 4 | | |
| *H. physodes* | 4 | 10 | 6 | 7 | 3 | 6 |
| *H. tubulosa* | 1 | 2 | 5 | 6 | < 1 | < 1 |
| *Menegazzia terebrata* | 1 | 2 | | | | |
| *Parmelia hygrophila* | | | | | < 1 | < 1 |
| *P. sulcata* | 1 | 2 | < 1 | 1 | 1 | 3 |
| *Parmeliopsis hyperopta* | | | | | < 1 | < 1 |
| *Platismatia glauca* | 70 | 100 | 301 | 181 | 44 | 41 |
| *P. herrei* | 7 | 16 | 33 | 41 | 3 | 4 |
| *P. stenophylla* | 91 | 345 | 127 | 141 | 1 | 3 |
| *Ramalina farinacea* | < 1 | < 1 | 1 | 2 | | |
| *Sphaerophorus globosus* | < 1 | 1 | 8 | 13 | 48 | 58 |
| *Usnea* spp. (tufted) | 7 | 16 | 4 | 6 | 1 | 2 |
| Bryophytes | | | | | | |
| *Antitrichia curtipendula* | | | | | 2 | 6 |
| *Dicranum fuscescens* | | | | | 10 | 46 |
| *Eurhynchium oreganum* | 1 | 4 | | | 2 | 6 |
| *Frullania nisquallensis* | | | | | 1 | 2 |
| *Hypnum circinale* | | | < 1 | < 1 | 9 | 36 |
| *Isothecium myosuroides* | | | 4 | 15 | 31 | 70 |
| *Neckera douglasii* | < 1 | < 1 | < 1 | 1 | 5 | 23 |
| *Orthotrichum lyellii* | < 1 | 1 | 1 | 3 | 2 | 6 |
| *Polytrichum juniperinum* | | | < 1 | < 1 | | |
| *Porella navicularis* | 1 | 6 | 4 | 16 | 11 | 24 |
| *Scapania bolanderi* | | | | | 5 | 20 |
| *Ulota crispa* | | | < 1 | < 1 | | |
| *U. megalospora* | | | | | < 1 | < 1 |

Litter data may also be used to estimate biomass of individual species rather than functional groups (Table 1, showing results for untransformed data). Although we have no independent assessment of the accuracy of these values, after square-root transformation the size of the standard deviations relative to the means is similar for most species to that of the functional groups as a whole, suggesting that the quality of the estimates would be comparable to the regression statistics reported above.

### SAMPLE SIZE FOR LITTER

A standard error of 10% of the mean for each functional group of lichens requires 9–27 of these plots (high end in old growth). If that rather stringent criterion for sample size is relaxed to 20%, then ten 2-m-radius plots would normally be sufficient for stands with simple structure and 15 plots for older stands with more complex, variable structure. In practice, this requires a single person ca. 4–8 hours of field time per stand.

Because litterfall is inherently very patchy, it is expected that long narrow rectangular plots would result in lower standard errors than circular plots, assuming equal plot areas. Although slower to lay out than circular plots, the higher statistical efficiency of rectangular plots may outweigh their slower setup.

## YEAR-TO-YEAR VARIATION

An important concern is the extent to which epiphyte litterfall, and hence the relationship between epiphyte biomass and epiphyte litter, varies from year to year. Our two years of data did not differ greatly in the total amount of litter. Some groups had higher biomass of litter in the first year, while others had higher biomass of litter in the second year. Clearly, a major storm will result in an anomalous pulse of litter which must be avoided, if one is to apply regression equations developed from years of normal litterfall. The importance of year-to-year variation can be minimized in several ways: 1) sampling at the end of long periods of nonviolent weather (late summer and early fall in the Cascade Range) to avoid large pulses of litterfall; 2) sampling the same stands in more than one year and combining results across years; and 3) using an adequate sample size to minimize noise from spatial variation in litterfall. Based on half-lives of litterfall determined for the Cascade Range area (McCune & Daly 1994), most of the pulse of winter litter should have disappeared within six months.

### LIMITATIONS AND USES

Despite all of the imaginable problems in applying this method, the results of this study demonstrate that, with a sufficient sample size, the late-summer epiphyte litter is reasonably representative of the epiphytes in the canopy in the area studied. Thus it appears that litterfall sampling can be used fruitfully for estimating at least orders of magnitude of epiphytic lichen biomass in tall forests, especially for lichens. Although the absolute biomass estimates will depend somewhat on time of sampling and year-to-year variation, the method should produce useful comparisons of relative abundance of epiphytes. The method is not recommended for bryophytes because bryophyte litterfall is highly aggregated, bryophyte litter is often difficult to distinguish from bryophytes in residence on logs and the forest floor, and because we have insufficient evidence of a consistent relationship between bryophyte litterfall and biomass in the canopy. The method is, however, recommended for large-scale surveys of lichens and other studies where large differences in epiphyte biomass are expected.

## ACKNOWLEDGMENTS

I am greatly indebted to my field and lab helpers: Bill Daly, John Davis, Ann DeBolt, Shannon Fitzpatrick, Jeff Green, Tonya Kocher, Roxy Johnston, Jeri Peck, Roger Rosentreter, Alice Smith, Bibit Traut, Jason Traut, and Laura Wilson. Stand and tree data and other assistance were provided by Mark Easter, Tom Spies, and other cooperators in a study of processes in forest gaps funded by NSF (BSR 8909038) to T. Spies, J. Franklin, and C. Vogt. Jeri Peck and Dan Norris assisted with identification of bryophytes. I appreciate the comments and advice of Bill Denison, Per-Anders Esseen, Patricia Muir, Fred Rhoades, Steve Sillett, Susan Stevenson, and anonymous reviewers. The Oregon State University Research Council provided partial support for this research.

## LITERATURE CITED

ANDERSON, L. E., H. A. CRUM & W. R. BUCK. 1990. List of the mosses of North America north of Mexico. THE BRYOLOGIST 93: 448-499.

EGAN, R. S. 1987. A fifth checklist of the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada. THE BRYOLOGIST 90: 77-113.

ESSEEN, P.-A. 1985. Litter fall of epiphytic macrolichens in two old *Picea abies* forests in Sweden. Canadian Journal of Botany 63: 980-987.

MCCUNE, B. 1993. Gradients in epiphyte biomass in three *Pseudotsugu-Tsuga* forests of different ages in western Oregon and Washington. THE BRYOLOGIST 96: 405–411.

——— & W. J. DALY. 1994. Consumption and decomposition of lichen litter in a temperate coniferous rainforest. Lichenologist 26: 67–71.

PERRY, D. R. 1984. The canopy of the tropical rain forest. Scientific American November: 138-147.

PIKE, L. H., W. C. DENISON, D. TRACY, M. SHERWOOD & F. RHOADES. 1975. Floristic survey of epiphytic lichens and bryophytes growing on living, old-growth conifers in western Oregon. THE BRYOLOGIST 78: 391-404.

———, R. RYDELL & W. DENISON. 1977. A 400-year-

old Douglas-fir and its epiphytes: biomass, surface area and their distributions. Canadian Journal of Forest Research 7: 680-699.

———, D. M. TRACY, M. A. SHERWOOD & D. NIELSEN. 1972. Estimates of biomass and fixed nitrogen of epiphytes from old-growth Douglas-fir, pp. 177–187. *In* J. F. Franklin et al. (eds.), Symposium proceedings: Research on Coniferous Forest Ecosystems, Bellingham, Washington, March 23–24, 1972. Northwest Scientific Association.

STEVENSON, S. K. & J. A. ROCHELLE. 1984. Lichen litterfall- its availability and utilization by black-tailed deer, pp. 391–396. *In* W. R. Meehan et al. (eds.), Fish and Wildlife Relationships in Old-growth Forests: Proceedings of a symposium held in Juneau, Alaska, 12-15 April 1982. American Institute of Fishery Research Biologists.

STOTLER, R. & B. CRANDALL-STOTLER. 1977. A checklist of the liverworts and hornworts of North America. THE BRYOLOCIST 80: 405-428.

WAFUNG, R. H., H. R. HOLBO, R. P. BUEB & R. L. FREDRIKSEN. 1978. Documentation of meteorological data from the coniferous forest biome primary station in Oregon. USDA Forest Service, General Technical Report PNW-73, Portland.