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April 1981

J.P. Blakeman
Aberdeen

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MICROBIAL PRODUCTIVITY ON AERIAL PLANT SURFACES

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INTRODUCTION

When the functional roles of various groups of organisms within an ecosystem are assessed, ecologists commonly use productivity, or rate of production, as a measure of relative importance. These measurements customarily involve monitoring some process directly associated with fluxes of energy and carbon such as release or uptake of gases during photosynthesis and respiration. Alternatively, changes in the biomass of a population can be estimated over a suitable interval. Strictly speaking, only phototrophs are capable of direct interception of solar radiation, the major input of physical energy to most ecosystems. Some ecologists would reserve the term "production" to such primary fixation of carbon and refer to the total energy and carbon flux through heterotrophic levels as "assimilation" (Odum, 1971). The heterotrophic assimilation of carbon is also commonly referred to as "secondary production", a term which explicitly recognises photosynthesis and respiration as separate facets of carbon flow through an ecosystem. Because comparisons of carbon fluxes through various compartments of an ecosystem are often of interest, I have preferred the looser construction of the term "production".

Quantitative studies on the ecology of epiphytic microorganisms in terrestrial habitats have seldom used measurements which permit any conclusions about rates of microbial production. For example, a survey of papers published in symposium volumes from the previous two meetings in this series (Preece and Dickinson, 1971; Dickinson and Preece, 1976) reveals 15 in which plate counts were used as a measure of microbial abundance and 6 where frequency of occurrence in

a sampling unit was used. Several authors employed other measures such as hyphal length or direct cell counts on the substrates. Only one (Collins, 1976) provided estimates of changes in microbial cell volume over time, measurements which could be extrapolated to give estimates of microbial productivity on the leaf surfaces investigated. Witkamp (1973) has provided evidence, both from microcosm studies and field data, that plate counts may be highly correlated with processes such as CO^2 evolution and substrate weight loss, which relate directly to microbial productivity. However, the relationships found for microbial communities in the soil may not apply to microbial communities on plant surfaces; it seems likely that such correlations may need to be determined anew for each distinctive habitat. In that case the data in the existing literature provide little information about the flow of fixed carbon and other elements through microbial populations in canopy habitats.

The paucity of such information can be explained easily. As noted above, microbial productivity is most readily monitored by measuring fluxes of gases associated with the metabolism of fixed carbon. Micro-organisms on plant surfaces typically occur as a fine skein of intertwined cells on leaves or bark (see e.g. Bernstein and Carroll, 1977). The microbial cell mass is usually small by comparison with the leaf or twig on which it occurs. For example Carroll (1979) and Swisher and Carroll (1980) have demonstrated microbial cell masses in the range of 10-20 µg dry weight on single Douglas fir (*Pseudotsuga menziesii*) needles, structures which themselves weigh between 2 and 4 mg each. Clearly the fluxes of respiratory and photosynthetic gases from the needles will prove far greater than those from the micro-epiphytes on the needles. We are thus confronted with the problem of measuring the respiration of a "hidden flea" on an elephant's back, one which cannot be found or removed.

The situation is not always so bleak. For example, Turner (1975) has measured the uptake of labelled CO^2 by the epiphytic alga *Pleurococcus* growing on twigs of European larch (*Larix decidua*) in Great Britain. The surface of the bark was scraped with a razor blade, releasing fragments of bark, fungal mycelium, and an algal powder. Since the algae were the only photosynthetic organisms in this mixture and since extracted chlorophyll was used to estimate algal abundance, the presence of other contaminating material made no difference to the uptake of labelled carbon nor to estimates of algal productivity. This approach may prove useful in other situations where investigators wish to estimate energy flux through populations of photosynthetic micro-epiphytes

(e.g. algae or crustose lichens) on non-photosynthetic surfaces. However, it will prove of little use for studies of secondary productivity on photosynthetic surfaces, i.e. for estimation of bacterial and fungal productivity on leaf surfaces.

If *in situ* process measurements are unavailable to the phylloplane microbiologist, other stratagems must be used to estimate productivity. Ecologists dealing with large organisms, particularly vascular plants, often find it convenient to estimate the biomass or standing crop of a population at two different times and to consider biomass increases over the chosen interval as a measure of production. Newbould (1967) has provided an exemplary formulation of these methods. His box diagrams emphasise the necessity for estimating losses due to shedding of plant parts and consumption or decomposition by heterotrophic organisms in addition to the apparent growth increment if a true estimate of productivity is to be obtained. Although this approach is used most frequently with higher plants, in principle it can be applied to any population of organisms provided that: (1) standing crops can be easily measured; (2) losses due to shedding, decomposition, and consumption are modest by comparison with the apparent growth increment; (3) the interval over which a biomass increment is measured is commensurate with growth rate; if the interval is too short, errors in measuring the increment will be unacceptably large; if the interval is too long, the growth of evanescent species in the population and random events, such as the death of whole trees may be missed entirely.

In practice precise comparisons of biomass before and after an interval of growth are seldom possible with field populations of micro-organisms. Microbial standing crops may be difficult to determine in field samples. Micro-organisms may grow opportunistically and rapidly during brief, unpredictable intervals when conditions are favourable; during the remainder of the sampling period they may not grow at all. Such erratic behaviour makes selection of an appropriate sampling interval difficult and may lead to unacceptably high statistical variance in the raw data. Finally, losses due to the death and shedding of biomass and to the activities of grazers and other heterotrophs may be very large by comparison with the standing crop. For example Nagel-de-Boois and Jensen (1971) estimated a relative annual production of 1000% (i.e. an annual production equal to 10 times the mean standing crop) for soil fungi in a temperate deciduous forest in the Netherlands. Flanagan and Bunnell (1976) calculated a relative annual production of 1500% for fungi inhabiting

standing dead leaves and stems of *Eriophorum* in a tundra habitat. In such situations it becomes more important to estimate death and shedding of biomass and losses to grazers and other heterotrophs accurately than to measure the biomass itself accurately.

A survey of a very scattered literature suggests that all of the general considerations noted above apply to microbial populations on aerial plant surfaces. Epiphytic microbial standing crops are at least as difficult to quantify as those in soil or litter. Cells are shed and exported from the canopy as micro-litterfall. Canopy micro-arthropods as well as smaller invertebrates may graze heavily on canopy micro-organisms. Although quantitative evaluation of microbial standing crops and biomass losses in canopy habitats may be difficult and uncertain, the approach offers some hope of approximating microbial production on aerial plant surfaces. Consequently, each of these considerations is discussed in more detail below with particular reference to canopy micro-epiphytes.

STANDING CROP ESTIMATION

Methods

Macauley and Waid (1981) have reviewed the methods available for determinations of fungal standing crops on aerial plant surfaces, and a simple repetition of that information is not proposed here. Generally these techniques fall into three categories: those involving visual measurements; those in which concentrations of various cellular components are determined chemically; and those in which certain cellular activities are measured. Each of these approaches involves significant inherent difficulties. Visual measurements of microbial cell volumes must be made under the microscope; they are extremely laborious and often yield uncertain data in a form unsuitable for easy statistical analysis (Visser and Perkinson, 1975; Bernstein and Carroll, 1977; Carroll, 1979; Carroll *et al.* 1980). Chemical estimators of microbial biomass (e.g. ATP, hexosamine, and ergosterol) assume a relatively constant ratio between the estimated chemical component and total biomass from which the component was extracted. Where that assumption proves unfounded, the values obtained can be used only as an index of biomass, not an absolute measure of it. In many cases micro-organisms grown in pure culture, not those from natural habitats must be used for standardising the assay. Several of these techniques (ATP, ergosterol) require expensive pieces of equipment and experienced laboratory personnel. The measurement of microbial metabolic activities (e.g. respiration, photosynthesis, dehydrogenase activity) requires first the discovery of some activity characteristic of the micro-organisms but not of the plant surfaces upon which they occur. Beyond this, such techniques may be blind to living, but inactive cells such as spores or quiescent vegetative cells.

Two methods omitted from Macauley and Waid's review circumvent many of the problems discussed above by combining a visual or chemical estimation procedure with a direct gravimetric determination of microbial cell mass from field-collected samples. New (1970) in studying the relative abundance of microbivorous Psocoptera on several species of trees in Great Britain found that microbial cells and other adventitious material on leaf surfaces could be efficiently scraped off with polystyrene chips. Electrostatic forces associated with the polystyrene assured virtually quantitative adherence of such materials to the scrapers. Consequently, when the chips were first tared, then used to scrape a known area of leaf surface, and finally reweighed, the difference in weights provided estimates for the total amount of material removed. Collodion peels were examined under the microscope to estimate the proportion of adventitious material falling into various categories: fungal hyphae and spores; algae; pollen; animal remains; and "other".

Swisher and Carroll (1980) have described a technique based on the release of free fluorescein dye when active microbial cells are incubated in a solution containing fluorescein diacetate. The fluorescein is determined spectrophotometrically by measuring the absorbance of solutions in which samples have been swirled at λ = 490 nm. The method was developed and first used successfully to estimate microbial biomass on needle and twig surfaces in a Douglas fir canopy. Materials released from needle surfaces by swirling served as gravimetric standards. Swisher and Carroll suggest that the method may show wide applicability wherever certain basic requirements can be met. In particular, the microbial cell mass must show the appropriate esterase activity, while the substrate itself must be inactive. Further, the substrate should not leach substances which absorb light strongly at 490 nm and which thus interfere with the spectrophotometric determination of the released dye. Finally, the fluctuations in biomass/activity ratio for the microbial cells must be acceptably small and satisfactory gravimetric standards must be used in which the physiological state of the "standard" cells resembles that of the microbial population to be determined. The importance of this last requirement is emphasised by more recent data from my laboratory (G. Carroll, unpub. obsv.)

which show that while biomass/esterase activity ratios are rather constant for microbial cells on dry canopy surfaces collected during the summer months, they may fluctuate greatly if the canopy has been previously wetted by rainstorms of any duration.

Where the above requirements can be met, this biomass assay offers several advantages: (1) the rates of fluorescein diacetate hydrolysis are linear with time over a great range of optical densities, and as a result, standing crops differing by as much as several orders of magnitude can be determined; (2) the method is simple and inexpensive; thus, a large sample size and multiple determinations can in part compensate for the imprecision of single determinations; (3) the assay is non-destructive and allows repeated measurements of microbial cell mass on the same substrate over a period of time.

Given the difficulty of estimating microbial standing crops on even small amounts of leaf or twig surface, it is scarcely surprising that such data have seldom been extrapolated to stand levels and expressed in terms of kg dry weight/ha, units which would allow direct comparisons with other components of an ecosystem. Firstly, the primary data are often highly uncertain (see e.g. Carroll *et al.*, 1980). In addition, microbial standing crops are usually expressed in terms of biomass or volume/unit surface area of leaf or twig; extrapolations to stand levels are thus very sensitive to errors in estimates of total leaf and twig area/ha of vegetation. Canopy micro-epiphytes have seldom been studied in forests where leaf area index and twig surface areas have also been determined. As a consequence, extrapolations to stand level must be preceded by a list of explicit assumptions and should usually be considered merely as order-of-magnitude estimates. As such they provide information on whether a given population of micro-epiphytes might be of significance in an ecosystem and thus worthy of further study. None of the studies published to date have estimated micro-epiphyte standing crops with anything near the accuracy with which the standing crops of vascular plants have been determined in similar studies.

Comparison of Estimates

The above notwithstanding, the meagre data available deserves some discussion. New (1970) demonstrated weights of adventitious material scraped from adaxial surfaces of leaves of several common forest trees in Great Britain ranging from 1-25 mg (100 cm^2)$^{-1}$. The median values for maximum standing

crops (during July) were seen on *Fagus* leaves and amounted to 6 mg (100 cm^2)$^{-1}$. Leaf area indices for temperate forests similar to those studied by New often fall in the range of 6-7 (Satoo, 1970; Heller, 1971). If a leaf area index of 6.5 is used and if a standing crop of 6 mg (100 cm^2)$^{-1}$ is considered typical, maximum standing crops of approximately 40 kg ha^{-1} are calculated. For several reasons these estimates are probably high. Firstly, the weights reported are not oven-dry weights. If the ratio of dry weight/fresh weight is assumed to fall in the range of 0.6-0.9 g g^{-1} reported by Carroll (1979) for micro-epiphytes on Douglas fir needles, estimates based on New's data would be reduced to 24-36 kg ha^{-1}. Second, not all the material weighed consisted of microbial cell mass. On *Fagus* and *Quercus* leaves, here considered typical, between 10% and 25% of the material weighed consisted of pollen, animal remains, and other non-microbial materials. The appropriate correction would reduce micro-epiphyte standing crops even further to 18-32 kg ha^{-1}. Finally, New's measurements were all taken from leaves in the lower part of the canopy. Carroll (1979) has shown that the density of micro-epiphytes on needles from the middle and upper strata of a Douglas fir canopy to be only half of that on needles from the lower canopy. If the same is true in the forests studied by New, estimates derived from his data should be further reduced. On the other hand, New did not measure micro-epiphyte standing crops either on the abaxial sides of leaves or on twig surfaces. Carroll (1979) and Carroll *et al.* (1980) have shown that both categories of surface contribute substantially to the total micro-epiphyte standing crop in a Douglas fir forest. Thus, any extrapolation from New's data to stand-level estimates must be, at best, an educated guess. In all probability, maximum micro-epiphyte standing crops for the forests he studied lie between 20 and 50 kg ha^{-1}.

Turner and Broadhead (1974) have studied populations of micro-epiphytes on leaf surfaces in a tropical evergreen forest in Jamaica, again in relation to populations of microbivorous Psocoptera. The cell volumes of fungi, algae, and crustose lichens on leaf and twig surfaces were estimated visually. The authors examined samples of mango leaves (*Mangifera indica*) from sites at intervals of 500 ft in elevation from 500-4000 ft. Although the data were presented in the form of volume indices, which cannot be used directly to estimate micro-epiphyte standing crops, a careful reading of the Methods section of their paper allows one to regenerate the raw data. If these forests are assumed to be similar to those of El Verde, Puerto Rico, a leaf area index

of 6.4 can be assumed (Odum, 1970). If cell volume/dry weight conversion factors are taken to be 0.7-1.1 g cm^{-3} (Carroll, 1979), these data can be expanded to yield estimates of microbial biomass in kg ha^{-1} (Table 1). While estimates from the lower elevation sites (500-1500 ft) fall into the range of standing crops derived from New's study, those from upper elevation sites are as much as two orders of magnitude larger. It seems unlikely that standing crops of epiphytic micro-organisms reach 2-3 tonnes ha^{-1} in any ecosystem. Two sources of error probably cause these inflated estimates. Thickness of microbial cells on leaves from lower sites was measured on collodion peels under the microscope and generally fell in the range of 5-10 µm, presumably the thickness of single cells. At upper elevation sites sooty moulds subsisting on insect honeydew formed mats up to 100 µm thick on leaf surfaces. These were measured in free-hand sections of the leaf under a microscope as the distance from the top of the mat to the leaf surface. In fact such mats are spongy and contain a great deal of free air-space, volume which is counted as microbial cell mass. Further, the samples were all taken from the lower portions of the tree crowns. Reynolds (1972) has shown that the distribution of fungi on leaves in a similar tropical evergreen forest is strongly skewed, with most of the biomass at the bottom of the canopy. Since the scheme for sample selection in this study was strongly biased, no firm conclusions can be drawn about the entire canopy. The estimates from higher elevations are probably at least an order of magnitude too high. Nevertheless, in any situation where standing crops of fungal epiphytes even approach 1 tonne ha^{-1} they probably play a highly significant role in carbon and mineral cycling within the system.

Finally, Carroll (1979) and Carroll *et al.* (1980) have published estimates of fungal standing crops on needles and twigs in an old-growth Douglas fir forest. In both studies the estimates were based on measurements of cell volume. Because portions of the canopy were sampled according to an unbiased, predetermined scheme and because the trees chosen for sampling had previously been completely described such that age-specific estimates for needle and twig surface areas were available (Pike *et al.*, 1977), these data probably represent the best available for stand-level estimates. Swisher and Carroll (1980) have recently estimated standing crops of the same microbial population chemically and found close agreement with the values obtained in the previous studies. Micro-epiphyte biomass on coniferous needles in such old-growth stands is estimated to lie between 38 and 60 kg ha^{-1} and is composed largely of fungi and algal cells.

TABLE 1

Stand level estimates of micro-epiphyte standing crops in evergreen forests of Jamaica. Estimates are based on measurements of microbial cell volume on leaves of Mangifera indica *by Turner and Broadhead (1974). All estimates are in units of kg dry wt* ha^{-1}.

	ELEVATION (FT)							
	500	1000	1500	2000	2500	3000	3500	4000
Upper leaf surface								
Fungi	15-24	41-64	7-11	24-38	312-490	2247-3531	407-639	861-1350
Lichen						43-68	421-661	23-36
Algae							5.0-7.5	4-6.5
Lower leaf surface								
Fungi	3.5-5.5	4.0-6.0	2.5-4.0	4.0-6.0	30-47	500-786	66-103	76-119
Lichen						1-2	70-110	1-2
Algae							13-21	
Both surfaces								
Fungi	18.5-29.5	45-70	9.5-15	28-44	342-537	2747-4317	473-742	937-1469
Lichen						44-70	491-771	24-38
Algae							18-28.5	4-6.5
Total Micro-epiphytes	18.5-29.5	45-70	9.5-15	28-44	342-537	2791-4387	982-1542	965-1514

The biomass on surfaces of twigs less than 15 years of age is estimated to lie between 13 and 21 kg ha^{-1} and is composed almost exclusively of fungal cells.

How do standing crops of micro-epiphytes, which usually amount to between 50 and 100 kg ha^{-1} and which are often composed largely of fungal cells, compare with standing crops of micro-organisms in other terrestrial habitats? They are clearly small, perhaps insignificant, by comparison with the fungal biomass estimated to occur in soil and litter in several ecosystems: Fogel and Hunt (1979) estimate the biomass of free-living and mycorrhizal fungi in the floor of a young Douglas fir forest to be 20 tonnes ha^{-1}; Visser and Parkinson (1975) have calculated the biomass of fungi in aspen litter as 2.2-2.8 tonnes ha^{-1} wet weight (factors for conversion to dry weight were not provided). On the other hand estimates for micro-epiphyte standing crop are large when compared with the 3.0-4.5 kg ha^{-1} which Flanagan and Bunnell estimated in standing dead leaf tissue of *Eriophorum* in a tundra habitat. Whatever the estimates, comparisons of standing crops may be almost meaningless if turnover times are short. Since such is suspected to be the situation with micro-epiphytes, some discussion of factors which contribute to microbial turnover on aerial plant surfaces is in order.

SHEDDING OF MICROBIAL CELL MASS: MICRO-LITTERFALL

Field Measurements

Micro-epiphytes in canopies of perennial plants, particularly woody plants, occur on surfaces which are short-lived in comparison with the plants themselves. Where a substantial portion of the total canopy surface area is shed annually, corresponding losses in the micro-epiphyte population must occur. Canopies of deciduous forests, where all leaves are shed annually, represent the most extreme example of this situation. Thus, in oak or beech forests studied by New (1970) in Great Britain the relative annual production can be no less than 100% of the maximum standing crop on leaves, since the entire microbial standing crop on leaves is shed in the autumn each year. For evergreen canopies the situation is somewhat less extreme, since leaves are usually retained for several years. For example, some needles on old-growth Douglas fir trees may be retained for 8-9 yr, although needles aged 1-4 yr account for at least 70% of the total leaf surface area (Pike *et al.*, 1977); 20-25% of the leaf surface is shed and renewed annually. However, those older needles that are shed tend to have higher than the mean

standing crop of micro-epiphytes (Carroll, 1979). Data from both Pike *et al.* (1977) and Carroll (1979) suggest that 30-35% of the needle micro-epiphyte biomass is shed with needles annually. Similar arguments apply to twig surfaces, although the proportion of twig surface area lost to annual shedding is a smaller proportion of the total (Pike *et al.*, 1977).

Microbial cells are also shed independently of the plant parts on which they occur. Evidence for such shedding of microbial cell mass from canopies is largely anecdotal, and quantitative information is virtually non-existent. For fungi cell mass may be exported from plant surfaces as spores. The literature of plant pathology is replete with references to spore release and dispersal of plant pathogens within crop canopies. Unfortunately, none of this literature provides information on the biomass of spores shed nor on the area of the canopy from which they originated. Bandoni (1981) and Carroll (1981) have both noted the presence of spores from aquatic hyphomycetes in throughfall from forested tracts (see Figs 1,2). Since such spores do not occur in the incident rainfall, they are presumed to originate in the canopies through which the rain has trickled. Vegetative microbial cells may also be shed from aerial plant surfaces. Reynolds (1975) has noted that entire sooty mould colonies on evergreen leaves in the lowland tropics are shed annually. Carroll (unpub. obsv.) has observed algal cells, *Atichia* colonies and sooty mould trichomes in the micro-particulate fraction filtered from throughfall (Figs 3-5).

Attempts to obtain quantitative estimates of microlitterfall (of which microbial cells may be a major component) are few and crude. Pike (1971) in a study of lichen production in a Willamette Valley oak forest, fitted 5 litter traps with foam rubber pads to trap "dust" (litter which would pass through a 1.3 mm mesh screen). He estimated 161 kg ha^{-1} of such material in the litter collected between July 31, 1968 and July 31, 1969. Since this fraction contained fragments of larger litter, pollen, insect parts, and other non-microbial materials, this must be considered merely an upper limit for microbial litter in this forest.

Perkins and Carroll (unpub. obsv.) attempted to estimate micro-litterfall in an old-growth Douglas fir forest in Oregon by taking cumulative samples of dryfall less than 1 mm in diameter during the dry summer months and collecting episodic samples of throughfall during single rainstorms in the fall, winter, and spring months. All samples were weighed on previously tared Nuclepore filters (0.2 µm pore size). The estimate for total annual dryfall amounted to

Fig.1. Spore of Ceratosporium cornutum *filtered from throughfall.*
Fig.2. Spore of Tridentaria *sp. filtered from throughfall.*
Fig.3. Algal cells and a colony of yeast-like cells filtered from throughfall.
Fig.4. Atichia *colony filtered from throughfall.*

45 kg ha^{-1}. The micro-particulates in throughfall are much more difficult to estimate. If cumulative samples are taken, the micro-organisms continue to grow in the throughfall collections during the sampling period, and in time form slimy layers on the inner surfaces of the collectors which cannot be removed quantitatively. If, alternatively, samples are collected at short intervals during single storms and filtered immediately in the field, accurate estimates of the micro-particulate fraction are assured. However, individual rainstorms may last several days: intensive field sampling during such a long interval has proved a practical impossibility. Concentrations of micro-particulates in throughfall change greatly during the courses of individual rainstorms as well as seasonally (Perkins, Horstmann and Carroll,

unpub. obsv.). Consequently, extrapolations of data obtained from short intervals of single rainstorms will yield highly uncertain estimates of annual totals.

Fig.5. Sooty mould trichome filtered from throughfall.

Episodic samples of throughfall were taken from 40-50 collectors over 1-2h intervals during 21 individual rainstorms which occurred between August, 1973 and June, 1975. When means of micro-particulates in throughfall from all samplers were computed, they varied among all storms by over an order of magnitude (from 0.12 kg $ha^{-1}cm^{-1}$ in the spring of 1975 to 2.9 kg $ha^{-1}cm^{-1}$ in the first August rains of 1973 and 1974). When the mean micro-particulate load in throughfall for all storms is extrapolated to an annual total on the basis of mean annual rainfall, a value of 250 kg $ha^{-1}yr^{-1}$ is computed. This value is almost certainly high, since the large amounts of micro-particulates found in early fall rains are atypical and since such storms were sampled with disproportionately high frequency. Extrapolation on the basis of the median value for all storms probably gives a less biased result, 130 kg $ha^{-1}yr^{-1}$. The total estimated annual micro-litterfall (dryfall + wetfall) thus amounts to 175 kg $ha^{-1}yr^{-1}$, a figure remarkably similar to that reported by Pike (1971) for an oak woodland.

Again, the above estimate represents an upper limit for shed microbial cells, since a large portion of the micro-litter, particularly the dryfall, consists of dust, bits of wood, pollen, and insect parts. Micro-particulates in

throughfall do, in fact, consist largely of microbial cells, and thus the true value for shedding of microbial cells from this canopy is unlikely to be less than 100 kg $ha^{-1}yr^{-1}$. If the standing crop of micro-epiphytes on needle and twig surfaces is taken to be 45 kg ha^{-1} (Carroll *et al.*, 1980), micro-litterfall would thus account for a relative annual production of 200-300%, with the entire standing crop turning over 2-3 times a year.

Microcosm Studies

A second approach towards monitoring the shedding of microbial cells from a canopy entails the use of laboratory microcosms. Carroll (1980) has described results from a series of experiments in which the output of dissolved and particulate nitrogen from a variety of canopy components (e.g. living needles and twigs, dead twigs, moss bolsters, chlorophycophilous lichens, and cyanophycophilous lichens) was monitored during simulated rainstorms in the laboratory. In summary, samples were taken from the canopies of old-growth trees permanently rigged for climbing (Denison, 1973; Figs 6,7) and were brought into the laboratory and stored overnight at 4°C. The following day samples were picked clean of extraneous material, placed in funnel assemblies, and misted for varying intervals at 16°C with previously collected rainwater which had been stored in a frozen condition until just prior to use. After misting, leachates were filtered sequentially through a 30 µm nylon mesh and then through tared Nuclepore filters with a 0.2 µm pore size. Aliquots of the filtered solution were taken for cation, total dissolved solids, and total nitrogen determination; the remainder of the fitrate was lyophilized, reconstituted to 10 ml, and stored frozen at -20°C for subsequent analyses. The microparticulates on the Nuclepore filters were dried at 80°C and weighed; a sub-sample of the filters was digested and analysed for total nitrogen.

Preliminary studies in which canopy samples were collected in the field every two weeks and misted in the laboratory for 1h revealed several striking trends: (1) fluxes of dissolved nitrogen were high when samples had been exposed to substantial rainfall (more than 2 cm) in the tree during the preceding two weeks. Examination of the filters from such misting experiments showed that the micro-particulate fraction from cyanophycophilous lichen thalli consisted largely of bacterial cells, while that from needles and twigs contained large numbers of algal and fungal cells. The differences in leaching patterns evident between "dry" and

Fig.6. Climber in seat-sling and stirrups preparing to ascend an old-growth Douglas fir tree.

Fig.7. Climber approximately 30 meters above the ground in the canopy of an old-growth Douglas fir tree. Note fan-like branch system on the right. Pipe-like spar on the left supports manoeuvrable platforms which carry micrometeorological sensors.

"wet" misting episodes suggested that important changes in the microbial populations on the surfaces of canopy samples occur during the transition from a dry to a wet canopy.

In order to investigate these changes, nutrient and particulate fluxes from canopy samples were monitored during prolonged (6-72h) laboratory misting experiments. While results from individual misting episodes differed, depending on which canopy component was studied, the same general trend was observed for all components: an initial pulse of leaching released dissolved nitrogen into the rainwater: however, after a lag period uptake of soluble nitrogen commenced; nitrogen in particulate form was released throughout each experiment. With a dynamic pattern of this sort, the uptake of soluble nitrogen from the rainwater is roughly balanced by the output of particulate nitrogen. At this point nutrient exchanges seem to be mediated entirely by populations of surface micro-organisms, with the canopy components themselves scarcely involved in the process. The length of the lag period before an equilibrium is reached appears to depend largely on the doubling times of the surface micro-organisms. Thus, for *Lobaria oregana* (a cyanophycophilous lichen), where bacteria comprise the bulk of the surface microbial standing crop, nitrogen uptake commenced after 2-3h. With needles and twigs, where fungi and algae are dominant surface micro-organisms, uptake was not detected for 6-10h, depending on the weight of sample used.

These experiments were carried out specifically with reference to canopy nitrogen fluxes. However, since particulate nitrogen was estimated as a proportion of total dry weight of micro-particulates, the data can also be used to generate estimates of microbial production. Table 2 shows data derived from a prolonged misting experiment with foliage and twigs of age-classes 2-4 yr. Four different weight classes of sample (2.86 g, 5.32 g, 10.24 g and 20.86 g dry weight) were placed in funnels and were misted with rainwater for 72h. The smaller two weight classes were misted at a rate of 16 ml h^{-1} and the larger two weight classes at a rate of 91 ml h^{-1}. During the last 12h of the experiment misting rates were changed to 100 ml h^{-1} and roughly 1200 ml of leachate was collected beneath each sample and was processed as described above.

If certain assumptions are made, the data from this experiment can be extrapolated to yield crude estimates of annual microbial turnover due to shedding of cells in rainwater. These assumptions include: (1) standing crops of micro-epiphytes are the same at the beginning and end of the misting episode; (2) biomass and surface areas of needles and twigs are apportioned among the 2-4 yr-old material in the samples as they are on the old-growth Douglas fir tree analysed by Pike *et al.* (1977); (3) volumes of micro-epiphyte cell mass/needle are as described by Carroll (1979); volumes

TABLE 2

Calculations of microbial turnover during 72h misting experiment with 2-4 yr twigs and foliage of Douglas fir.

	Weight Class I	Weight Class II	Weight Class III	Weight Class IV
Sample Dry Weight (g)	2.86	5.32	10.24	20.86
Est. Twig Microbial Vol. (mm^3)	1.52	2.83	5.46	11.13
Est. Needle Microbial Vol. (mm^3)	13.10	24.57	47.92	95.91
Est. Total Microbial Vol. (mm^3)	14.6	27.4	53.4	107.0
Est. Total Microbial Biomass (mg)				
Low conversion factor (0.7)	10.2	19.2	37.4	74.9
High conversion factor (1.1)	16.1	30.1	58.7	117.7
Total Measured Filterable Solids (mg)	1.72	2.55	23.5	54.1
Calculated Turnover (%)				
Low Est. Biomass	16.7	13.3	62.8	72.2
High Est. Biomass	10.7	8.5	40.0	46.0
Volume of Water Misted				
ml	2095	2103	6661	6732
cm "rain"	9.5	9.6	30.3	30.6
Average annual rain at field site (cm)	240	240	240	240
Number of Micro-epiphyte Generations Annually (1/Turnover Time)				
Low Est. Biomass	4.2	3.3	5.0	5.7
High Est. Biomass	2.7	2.1	3.2	3.6

of micro-epiphyte cell mass cm^{-2} twig surface area are as described by Carroll *et al.*(1980); (4) the density of micro-epiphytic cells lies between 0.7 and 1.1 g dry weight cm^{-3} (Carroll, 1979); (5) all micro-particulates filtered from water collected below the samples consisted of microbial cells. Presumed volumes of both needle and twig micro-epiphytes are shown in the upper portion of Table 2. Below this, corresponding microbial biomass estimates appear for each weight class of sample. Two such estimates are shown for each sample, one supposing a low volume/weight conversion factor (0.7) and the other supposing the higher value for the conversion factor (1.1). Microbial turnover during the experiment was calculated by dividing the observed weights of filterable solids by the presumed mean microbial standing crop for each sample. Since the percentage turnover depends on standing crops, two estimates are presented for each weight class, corresponding to the low or high volume/weight conversion factors mentioned above. When the total volume of water collected beneath each sample is divided by the surface area of the collector funnel, the amount of water intercepted by the sample can be expressed in cm of rain. The mean annual rainfall at a meteorological station several miles from the site where the canopy samples were collected is approximately 240 cm/yr. If the percentage microbial turnover computed for this laboratory misting episode is multiplied by the ratio of mean annual rainfall/laboratory "rainfall", a total annual turnover can be computed. Depending on which standing crop estimate is accepted and which weight class of sample is considered, estimates for annual turnover range from 2.1-5.7 times the mean standing crop. The dispersion of needles and twigs along single branches in a real canopy is most closely reflected in our experiment by the Weight Class II sample. Thus, an annual turnover of 2.1-3.3 represents the most reasonable extrapolation for shedding of cells in throughfall.

Crude as this estimate may be, it shows remarkable agreement with field measurements of micro-litterfall described above. If the entire micro-epiphyte standing crop of needles and twigs, estimated by Carroll *et al.* (1980) to be 45 kg ha^{-1} turned over 2.1-3.3 times/yr, the estimates for microbial cells shed in throughfall amount to 95-150 kg $ha^{-1}yr^{-1}$. The 100-130 kg $ha^{-1}yr^{-1}$ guessed from actual field measurements of micro-particulates in throughfall are bracketed nicely by these laboratory estimates. In future experiments it should prove possible to repeat such studies using the fluorescein diacetate technique to quantify standing crops on samples. With real data on standing crops at hand, a major source of uncertainty in the laboratory estimation of production by micro-epiphytes will be eliminated.

Computer Modelling Efforts

The laboratory and field studies described above all suffer from a fundamental debility; they are essentially descriptive, and as such they lack predictive power. The existing information about microbial productivity on aerial plant surfaces is tied to the particular vegetation type and set of climatic conditions for which it was gathered. No references from existing data are yet possible about micro-epiphyte productivity in different vegetation types under different climatic conditions. As a result, whenever a new ecosystem where micro-epiphytes may be important is studied, the same laborious and expensive empirical measurements must be repeated. This situation will persist so long as we lack basic information about the constraints on microbial growth in canopy habitats.

Computers simulation models in which information on growth parameters of canopy micro-organisms are integrated with data from microcosms and field measurements represent an approach towards solving this quandry. Such models predict how the micro-epiphyte population should grow under a variety of environmental conditions; usually temperature and relative humidity are of prime concern, although for photoautotrophs light intensity is also an important factor. When the seasonal distribution of optimal growth conditions in a canopy is known, successful simulation models can provide an integrated estimate of growth over an entire year, i.e. an estimate of microbial production.

To my knowledge only a single simple model of this nature has been published. Turner (1975) determined photosynthetic rates for *Pleurococcus*, a green alga occurring on dead bark of European larch, under varying conditions of light, relative humidity, and temperature in the laboratory. Linear multiple regression analyses showed that net primary productivity was strongly correlated with relative humidity, less strongly correlated with light levels, and not significantly correlated at all with temperature. In fact a relative humidity of 70% was required before any photosynthesis at all took place. Turner then extrapolated these results to field situations on the basis of previously collected micro-meteorological data. He concluded: (1) relative humidity in the vicinity of larch twigs seldom dropped below 60%; thus it was likely that relative humidity levels seldom limited algal photosynthesis in actual larch plantations and thus light levels were more important in the actual field situation;

(2) assuming a *Pleurococcus* cover on branches of 25%, a surface area of 20 m^2 for a typical small larch tree, several constants from the literature for photosynthetic efficiency, and an average value of 5-10 h day^{-1} over the whole year when climatic conditions allowed a production rate of 5 x 10^{-4} $gm^{-2}h^{-1}$, the likely annual primary production for *Pleurococcus* fell between 9 and 18 kg ha^{-1}.

Work still in progress at the University of Oregon by W.R. Massman, P. Sollins, and myself has included the development of a simulation model to predict growth, uptake of dissolved nitrogen from the canopy solution, and loss of microbial cells in the throughfall by micro-epiphytes during the course of single rainstorms in an old-growth Douglas fir forest. The model is structured so that the paramount role of surface micro-organisms in regulating fluxes of dissolved and particulate nitrogen from the canopy is recognised; it functions in a mechanistic fashion such that mass, conserved as nitrogen, is transferred from one reservoir to another. Nitrogen contained in incident precipitation and that available through washing or leaching from canopy components at the beginning of a storm comprise inputs to the system. Outputs of both dissolved and particulate nitrogen occur as rain falls through the canopy to the forest floor. In formulating the model the canopy has been divided into five vertically stacked strata such that the output from one stratum becomes the input to the next. Each stratum is considered to consist of three components whose surface microbial floras and behaviour with regard to nitrogen fluxes differ. These are: (1) chlorophycophilous lichens, which take up nitrogen into their thalli almost immediately upon wetting, which continue to do so throughout a storm, and which release a cellular particulate matter to the throughfall; (2) cyanophycophilous lichens and mosses, which initially leach large amounts of dissolved nitrogen, but later release nitrogen largely in particulate form as bacterial cells; (3) needles and twigs, which initially leach small amounts of dissolved nitrogen/g of sample but later release nitrogen in particulate form as fungal and algal cells.

The mathematical expressions for the model involve four basic types of coupled ordinary non-linear first order differential equations for each of the five layers. These four equations predict (1) the flow of water through the canopy; (2) the amount of soluble nitrogen in the canopy; (3) the microbial biomass; (4) the amount of intercellular nitrogen in the micro-epiphytes. The specific equations for (3) and (4) differ for each of the three components in the model. Use of equations in this form has made it simple to test model predictions and to make major changes without extensive reprogramming: terms from the appropriate equations are merely added or deleted.

In any model simplifying assumptions must be made. A number of assumptions have been made for this model. Several may not always apply and so should be explicitly stated before discussing model output and validation. These are: (1) inputs of particulate nitrogen in the rain are negligible and pools of particulate nitrogen in the canopy are entirely microbial; inputs and transfers of nitrogen in inert materials such as dust, wood particles, frass, and insect exuviae are not considered; (2) each stratum in the canopy is horizontally homogeneous; (3) microbial growth is non-colonial; (4) nitrogen is not lost from the system in gaseous form; (5) nitrogen alone limits microbial growth; (6) the flow of nitrogen into microbial cells is unidirectional, and losses of nitrogen through death and lysis of cells does not occur; (7) canopy eukaryotic micro-organisms take up nitrogen in excess of their immediate needs. Many of these assumptions either seem eminently reasonable or have been proved legitimate through laboratory observations. Certain of them may not apply under extreme conditions. Thus, although evergreen canopies are frequently net sinks for dissolved nitrogen during rainstorms, they are unlikely to behave so in situations where trees have been exposed to high levels of foliar fertilization prior to storms. Under such conditions the micro-epiphytes would surely become saturated with nitrogen, and some other element, probably phosphorus, would limit microbial growth. These considerations become very complicated if several elements simultaneously limit growth (Waite and Mitchell, 1972). Other assumptions are known to be incorrect. For example, the canopy of these trees is known to be heterogeneous at a fine level of resolution (Pike *et al.* 1977). However, at the coarser level of spatial resolution with which the model works, the assumption may be permissible in a statistical sense.

Before this model can be run, values for a number of parameters which relate to uptake of nitrogen and growth of surface micro-organisms must be supplied. These include: (1) the Michaelis-Menten half-saturation constant for various surface micro-organisms; (2) a number associated with the rate of removal of microbial cells from canopy surfaces; (3) the temperature-dependent specific growth rate for each category of canopy micro-organism (e.g. bacteria, fungi, algae); (4) the maximum specific uptake velocity of nitrogen for each category of canopy micro-organism; (5) the rate at which nitrogen becomes available for leaching from various

canopy components. It is expected that values for parameters which relate specifically to microbial growth will differ significantly between eukaryotic and prokaryotic micro-organisms. During the preliminary stages of model development we have either guessed at parameter values or taken them from the literature. In the near future we expect to compute actual values for these parameters, either from the microcosm studies described above or from experiments specifically planned for the purpose. We anticipate that measurement of these parameters in the laboratory will prove considerably less laborious than estimation of biomass and production in the field. In fact the robustness of simulation models lies in just this ability to predict output under field situations from laboratory measurements.

Although our model is still under development, preliminary output shows good qualitative agreement with observed patterns of nitrogen flux seen during laboratory misting episodes. Most significantly, the model predicts that, as the number of strata in the canopy increases, the efficiency of nitrogen uptake also increases, with the result that a larger portion of the total nitrogen released at the bottom of the canopy appears in particulate form. During prolonged misting episodes in the laboratory increasing numbers of strata have been simulated by placing increasing amounts of a given canopy component in a funnel. With both *Lobaria* and Douglas fir needles and twigs the effect has proved striking: total output of particulate nitrogen/g of sample increases and output of dissolved nitrogen/g sample decreases with increasing weights of sample/funnel. Further, uptake of nitrogen commences more quickly with increasing sample weight.

Only one preliminary field validation experiment has been attempted to date. Samples were taken from six $0.3m^2$ throughfall collection troughs placed at random beneath a single old-growth tree. Incident rainfall was collected in a single sampler in an adjacent clearcut. Samples were collected at hourly intervals for 4h at the beginning of a rainstorm; they were filtered in the field and stored on ice prior to analysis. Although control samples (incident rain) were not replicated, trends in fluxes of dissolved nitrogen were unequivocal: dissolved nitrogen was scavenged from the incident rain by the canopy throughout the portion of the storm that was monitored, a result predicted by our preliminary model. More recent runs of a more complete version of the model have predicted another effect. Movement of micro-particulates from top to bottom of the canopy is slow, a matter of hours. In our field experiment, after an initial flush of micro-particulates from the canopy, concentrations

in throughfall dropped and stayed near the control values. If we had monitored the storm for several more hours, the second pulse of newly generated micro-particulates predicted by the model might well have been seen. During storms of short duration the micro-particulate fraction may never be flushed from the canopy. Instead it may be left as a particulate residue as water evaporates from the tree, only to be washed out during the initial phases of the next storm.

The foregoing model has been presented in considerable detail because it is a process model of considerable generality which may prove applicable to other canopies than the one for which it was developed. In concluding this section several aspects of the model should be stressed. Although it specifically simulates nitrogen fluxes, the model also deals with microbial production, since actual weights of microbial cell mass must be computed at each step before nitrogen contents are calculated. Because of this structure, a suitably modified version of the model could deal with canopy fluxes of any macroelement in which microbial uptake during rainstorms is involved. Secondly, this model simulates nitrogen fluxes at a finely resolved scale during single storms. It does not deal with other processes which are known to affect the nitrogen status of the canopy such as nitrogen-fixation by *Lobaria oregana* or grazing by micro-arthropods. These processes operate more slowly, over longer periods, and should thus be modelled at coarser levels of time resolution. In principle sub-models could be constructed for each of the processes; they would, however, add considerable complexity to an already complicated model. Finally, the model deals only with single storms. If annual totals are desired, they must be computed on the basis of information on the number, duration, and seasonal occurrence of all rainstorms in a given year. When model output for each type of storm is available, appropriate summations will yield annual totals.

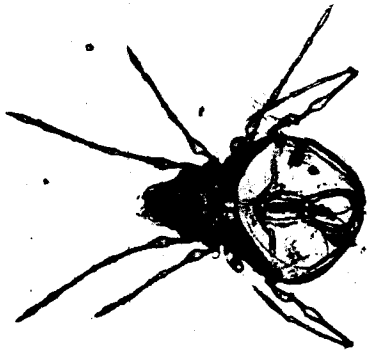
MICROBIAL CELL LOSSES DUE TO MICROFAUNAL GRAZING

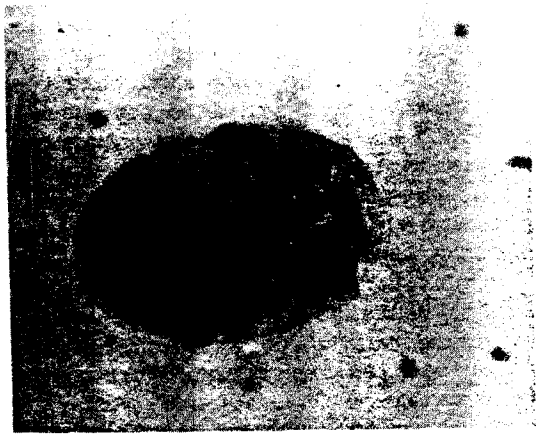
In the last 25 years a significant literature has accumulated on the grazing of canopy micro-epiphytes by micro-arthropods. Initial studies by Broadhead (1958) showed that large and diverse populations of Psocid grazers occur on the bark of European larch and that they subsist entirely on *Pleurococcus* cells, fungal spores and vegetative cells (believed to be *Sporidesmium scutellare*, but probably consisting of a number of species), and the crustose lichen *Lecanora conizaeoides*. Broadhead further showed that the nine most abundant species

partitioned this habitat very finely, both phenologically and nutritionally, with many species preferring, or even requiring a particular kind of microbial food. Indeed, a later paper (Broadhead and Wapshere, 1966) involving the same system has become a classic on the subject of habitat partitioning. More recent papers have shown that grazing of micro-epiphytes by Psocids is a general phenomenon. New (1970) examined foliage of 12 species of trees in Great Britain and found that leaves of all were inhabited by at least one or two species of microbivorous Psocoptera; several, such as *Crataegus monogyna* Jacq., *Quercus robur* L., and *Quercus petraea* (Mattuschka) Liebl. showed large and diverse populations. Similar populations of Psocoptera occur on leaves of *Mangifera indica* (Turner, 1974) and on leaves and twigs of *Juniperus lucayana* and *Podocarpus urbanii* (Turner and Broadhead, 1974). The microbial food in this latter study was determined to consist largely of sooty moulds, *Phycopeltis* cells (a green alga), and cells of some undetermined powdery lichen.

More recent studies have shown that micro-arthropods other than Psocoptera have exploited canopy micro-epiphytes as a source of food. Dr. David Voegtlin, while at the University of Oregon, carried out an extensive 30-month survey of arthropods in the canopy layer of an old-growth Douglas fir forest. He sampled foliage and small twigs intensively over a 12-month period. Canopy samples were collected every two weeks and processed in the laboratory by agitating vigorously in water and filtering through a graded series of small mesh screens. He found mites, including species of *Camisia, Phauloppia, Platyliodes, Scapheremaeus,* and *Scleroribates* to be extremely prevalent on foliage and living and dead twigs. André and Voegtlin (1981) have shown that *Camisia* is a fungivorous mite (Fig. 8). The other mites found are also suspected to graze on microbial cells (Fig. 9). Other known prevalent microbivores from this habitat include several species of Collembola and the larvae of a Dipteran fly, *Lestodiplosis* sp. Although adult Psocoptera were only rarely recovered from needles and twigs, Psocopteran eggs were extremely abundant in these samples, and large numbers of adults were caught in sticky screens suspended in the canopy. Several of the genera and even species were those found by Broadhead and his colleagues to graze on micro-organisms. Psocoptera probably also graze micro-epiphytes in the old-growth Douglas fir canopy, but were not recovered by Voegtlin in foliage samples because of an inappropriate sampling technique.

Microscopic observations of material recovered from various canopy components leave little doubt that grazing of microbial





Figs 8 and 9. Left: Frass pellet from immature stage of Camisia carrolli. *Note undigested remains of fungal hyphae. Right: Fungivorous mite from Douglas fir foliage.*

cells extends even to the level of ingestion of bacteria and other small-celled micro-organisms such as yeasts by microscopic invertebrates. Tardigrades and rotifers are common on needles and twigs (Voegtlin, unpub. obsv.). These animals are so small as to render ingestion of cells larger than a few microns impossible. Protozooans may also be involved in grazing microbial cells from canopy surfaces. Carroll and Pike (unpub. obsv.) have noted that bacterial populations on the surface of *Lobaria oregana*, the dominant lichen in old-growth Douglas fir crowns, become very reduced during the spring of each year. Bacteria never become prevalent on the surfaces of *Peltigera membranaceae* (Ach.) Nyl., a common soil lichen in old-growth forests of the Pacific Northwest. In both situations scrapings from the surface of lichens which have been moist for some time and micro-particulates which have been washed off the lichen surfaces contain large numbers of testate amoebae and cysts of such amoebae. These protozooans are presumed to account for the observed decline in bacterial standing crops.

How much microbial biomass might be consumed by a canopy microfauna annually? Unfortunately there is a dearth of quantitative data bearing on this question. Occasional comments in the literature suggest that the impact of small animals on micro-epiphyte standing crops may be substantial. Broadhead (1958) noted that twig segments of larch were frequently grazed bare of *Pleurococcus* by Psocids. Carroll (1979) observed that standing crops of micro-epiphytes in the upper crowns of Douglas fir trees were much lower than in the bottom layers of the canopy; he suggested that the elevated populations of *Camisia carrolli* André reported in the upper canopy by André and Voegtlin (1981) might account for this distribution. Similarly, the number of *Camisia* individuals/unit length of twig segment increases steadily from age-class 1 twigs to age-class 5 twigs, after which populations of mites decline (André and Voegtlin, 1981). This distribution may account for the drastic decline in needle micro-epiphyte cell volume on age-class 4 needles (Carroll, 1979). However, André and Voegtlin report that *C. carrolli* never feeds on needles, and thus, other mites or Collembola may be responsible.

Where standing crops of the microfauna are available, a crude estimate of grazing losses can be generated by assuming some mean conversion efficiency from microbial biomass to animal biomass. Broadhead (1958) reported 50 kg ha^{-1} of Psocoptera on twig surfaces in a larch plantation. Since all of the species studied produce only a single generation/yr, this corresponds to a production rate of 50 kg $ha^{-1}yr^{-1}$.

If the conversion efficiency of Psocids feeding on micro-organisms is 10%, 500 kg $ha^{-1}yr^{-1}$ of microbial food must have been eaten to account for that weight of insects. This is probably an overestimate. Firstly, Broadhead overestimated the density of larch trees and hence the density of Psocids in the plantation he studied. Turner (1974) suggests that a Psocid standing crop of 10 kg ha^{-1} may be a more realistic value for that plantation, a level requiring only 100 kg $ha^{-1}yr^{-1}$ in a microbial cell mass. Beyond this, many such insects are coprovorous. With each passage through the gut, some new portion of the original material is utilised, often through the action of associated bacteria (Hargrave, 1976). The net effect of such repeated ingestion would be an increase in the assimilation efficiency and a corresponding increase in the production efficiency (assimilation = production + respiration) beyond the commonly assumed value of 10%. On the other hand, an adult population of 10 kg ha^{-1} implies substantially more total Psocid production, since a majority of the young insects are probably devoured by predators or parasitoids. Voegtlin (unpub. obsv.) found large numbers of spiders and other predators in the canopy of an old-growth Douglas fir forest. The total annual consumption of microbial cell mass in the larch plantations studied by Broadhead is unlikely to be less than 50-100 kg ha^{-1}, an amount several times the mean standing crop of micro-epiphytes.

André and Voegtlin (1981) have provided estimates for the numbers of *Camisia carrolli* in a single Douglas fir tree. Although the highly aggregated distribution of the mites makes their estimates uncertain, the data suggest that at least 3×10^5 mites occur in a single tree. If there are the equivalent of 50 such trees/ha (Carroll, 1979), a value of 15×10^6 mites ha^{-1} is computed. Individual adults measure approximately 0.2 x 0.3 x 1.0 mm; the corresponding volume is approximately 0.06 mm^3 and 15×10^6 mites would have a volume of 9 litres and a corresponding weight of 3-9 kg. If their production efficiency is 10%, they would consume 30-90 kg of micro-epiphyte cell mass in generating that weight. The same arguments for underestimation mentioned above for Broadhead's data also apply here. Further, the computations on *Camisia* ignore the other equally numerous microbivores on needles and twigs of Douglas fir. It seems likely that the microfauna consumes many times the mean standing crop of micro-epiphyte cell mass in an old-growth Douglas fir canopy.

Studies on the energetics of the microfauna in soil and litter, admirably reviewed by Harding and Stuttard (1974), shed additional light on the data from canopies and suggest

productive avenues for further research. The methods developed to estimate consumption rates and production efficiencies for these soil and litter populations fall into several categories: (1) visual methods, in which mean standing crops of gut contents are estimated under the microscope and compared with observations on rates of production of faecal pellets (Mitchell and Parkinson, 1976); (2) gravimetric methods in which weights of food sources before and after feeding are compared with weights of resulting faecal pellets; (3) radiotracer methods.

Detailed repetition of the results cited by Harding and Stuttard (1974) is beyond the purview of this discussion. However, several general conclusions can be drawn. The mean densities for oribatid mite populations in soil and litter range from 67×10^6 - 1.34×10^9 ha^{-1}. These numbers are larger than the number of *C. carrolli* individuals/ha in a Douglas fir canopy; however, other oribatid mites are almost as numerous in this latter habitat, and in fact, the overall mite densities are probably comparable with those in soil and litter. Biomass estimates for terrestrial mites range from 3-54 kg ha^{-1} live weight; dry weight estimates are not available from Harding and Stuttard's review, but probably lie in the range of 1-20 kg ha^{-1}. Standing crops of canopy mites are probably comparable.

Assimilation efficiencies for soil and litter micro-arthropods lie between 10% and 20%. However, assimilation comprises two components: production and respiration. Several studies have dealt with the allocation of assimilated energy between production and respiration, with results varying between 5% allocation to production and 95% allocation to production. An intermediate perhaps more reasonable production/assimilation ratio of 27% was cited for the oribatid mite population of the IBP site at Meathop; the same study put the assimilation efficiency (assimilation/consumption) at 32%. The ratio of production/consumption is the product of these two ratios of 8.6%, somewhat lower than the 10% used for computations on canopy Psocid and mite consumption above.

Although numbers of micro-arthropods in the forest canopies and forest floor may be comparable, the total resources available to the forest floor populations are 10-100 times greater. Many arthropods in soil and litter feed on decomposing plant materials as well as micro-organisms. Since the supply of lodged litter in canopies is limited, canopy micro-arthropods depend almost exclusively on micro-epiphytes and micro-litter (e.g. pollen) as a food source. The consequent grazing pressure on canopy microbial populations must be substantially greater than in soil and litter.

All of these considerations suggest that the computations made in the early part of this section are conservative and that grazing by micro-arthropods may be at least as significant a factor in the turnover of micro-epiphyte biomass as the shedding of cells in throughfall and dryfall. If so, microcosm studies as well as field experiments on canopy micro-arthropods will be necessary before a complete picture of microbial production in any canopy emerges.

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STATISTICAL METHODS IN THE ANALYSIS OF PHYLLOPLANE POPULATIONS

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INTRODUCTION

A considerable body of information now exists concerning microbial populations on the phylloplanes of many plants (Last and Deighton, 1965; Preece and Dickinson, 1971; Last and Warren, 1972; Dickinson and Preece, 1976). Attempts to characterize these populations, in terms of abundance and diversity of taxa, have led to the development of various microbiological techniques. The two principal approaches used to investigate phylloplane populations are categorized as direct and indirect methods. Direct methods involve examination of the phylloplane *in situ* by light or scanning electron microscopy ('semi-direct' methods first attempt to incorporate the microbial propagules in viscid cellulose compounds or on transparent adhesive tapes). They provide useful data including growth forms and spatial distributions of a variety of microorganisms. However, problems associated with determining the viability and identity of propagules, as well as the tedious nature of the work, have restricted the use of direct methods for detailed quantitative studies.

Most investigations have used indirect methods of examination which involve attempts to remove microbial propagules from leaf surfaces to artificial media for subsequent growth, identification and enumeration. The results obtained require careful interpretation because many factors can influence the sample estimates of various microbial populations. It is necessary therefore to study the efficiency of removal of