Biomass and distribution patterns of conifer twig microepiphytes in a Douglas fir forest

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Total cell volume estimates and species composition for twig microepiphytes are presented for four species of coniferous trees common in the Pacific Northwest. Fungal cell volumes per square centimetre were much greater on *Pseudotsuga menziesii* than on the other conifers sampled; population densities of algal cells were similar on all hosts. On *P. menziesii*, microbial populations build up as the twigs age, with total cell volumes at a maximum on 6- and 7-year-old twigs; thereafter total cell volumes decline as twig surface areas decline, although population densities remain high. When fungal cell volumes are expanded to tree and stand levels, estimates of 372 cm^3/tree and 15 kg/ha are generated. These values are significant in comparison with estimated standing crops of epiphytic fungi on needles (30 kg/ha) and suggest that twig microepiphytes must be considered in any investigation on the role of epiphytes in nutrient cycling in coniferous forests.

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Les auteurs présentent des estimations du volume cellulaire total, ainsi que la composition spécifique des microépiphytes des ramilles de quatre espèces de conifères arborescents de la région pacifique du nord-ouest. Le volume de cellules fongiques par centimètre carré est beaucoup plus élevé sur *Pseudotsuga menziesii* que sur les autres conifères échantillonnés; la densité des populations de cellules d'algues est la même chez tous les hôtes. Chez *P. menziesii*, les populations microbiennes augmentent avec l'âge de la ramille et le volume total de cellules atteint un maximum sur les ramilles âgées de 6 et de 7 ans; ensuite, le volume total de cellules diminue avec la surface de ramilles, mais la densité des populations demeure élevée. Si l'on extrapole le volume de cellules fongiques jusqu'aux niveaux de l'arbre et du peuplement, on obtient des estimations de 372 cm^3/arbre et de 15 kg/ha respectivement. Ces valeurs sont importantes en comparaison avec la biomasse estimée de champignons épiphytes sur les aiguilles (30 kg/ha) et montrent que les micro-épiphytes des ramilles doivent être considérées dans toute recherche sur le rôle des épiphytes dans la circulation des éléments nutritifs dans les forêts coniférienes.

[Traduit par le journal]

Introduction

The needles and twigs of coniferous trees are known to harbor a diverse population of epiphytic microorganisms composed largely of fungi. Sherwood and Carroll (1974) have studied microbial succession on needles and twigs of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and have found a few dominant species in populations of microepiphytes. For needles these species include the following: *Aureobasidium pullulans* (De Bary) Arn., a ubiquitous surface colonist with pigmented mycelium and yeastlike conidia; *Atichia glomerulosa* (Ach. ex Mann) Stein (= anamorph of *Seuratia millardetii* (Racib.) Meeker), a loculoascomycete with a pseudoparenchymatous, nonmycelial growth habit which produces yeastlike cells in thin films of water (Meeker 1975); and *Prototococcus* sp., a unicellular green alga. On twigs several additional species of microorganisms are seen, notably representatives of the Fungi Imperfecti (*Epicoccum* sp., *Cladosporium* sp.) and several species of sooty molds, including *Antennatula* sp. in the Euantennariaceae and *Metacapnodium* sp. in the Metacapnodiaceae; these sooty molds are typical loculoascomycetes, the latter genus producing characteristic trichomelike mycelial filaments with tapering ends (Hughes 1976).

A description of microepiphyte distribution and estimates of total microbial biomass on the surfaces of Douglas fir needles in the canopies of two old-growth trees have recently been provided by Bernstein and Carroll (1977) and Carroll (1979). These studies have revealed the presence of populations which may account for the assimilation of

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TABLE 1. Site data on sampled trees

Tree	Species*	Trunk diameter at 1.3 m, cm	Total height, m	Site aspect	Site elevation, m	Association*	Branchlets sampled for microepiphytes: Branch system No.	Height above ground, m	Total twig surface area for branchlet, cm²
286	PSME	142	77	S	460	TSHE–ACCI–POMU	6	30	6154
							24	42	3664
							43	52	3206
1137	PSME	108	ca. 50	S	510	PSME–TSHE–CACH	62	43	3055
98	PSME	141	57	W	580	TSHE–RHMA–BENE	10	16	2964
							25	28	3668
							75	51	1543
778	PSME	15	12	W	530	PSME–TSHE–CACH	52	9	859
885	TSHE	26	21	WNW	480	TSHE–RHMA–BENE	39	6	1230
1169	TSHE	45	28	SW	530	PSME–TSHE–CACH	35	12	329
							97	22	247
914	TABR	24	9	WNW	530	TSHE–ACCI–POMU	82	7	634
1206	TABR	20	16	WNW	630	TSHE–RHMA–BENE	18	12	858
26	TABR	17	8	NW	650	PSME–TSHE–CACH	12	3	2390
705	PILA	122	55	WNW	590	TSHE–RHMA–BENE	106	31	493
							154	39	345
							267	50	22

*PSME, *P. menziesii*; TSHE, *T. heterophylla*; TABR, *T. brevifolia*; CACH, *C. chrysophylla*; RHMA, *R. macrophyllum*; ACCI, *A. circinatum*; POMU, *P. munitum*; BENE, *B. nervosa*.

substantial amounts of fixed carbon and nitrogen from canopy leachates. Although twig surface area is less than one-fifth of the needle surface area in a typical old-growth Douglas fir tree (Pike *et al.* 1977), casual visual inspection of twigs suggested higher microbial population densities on twigs than on needles. Consequently, the present study was undertaken to estimate densities of microepiphytes on twig surfaces. In addition to estimating standing crops, information on differences among several host trees with regard to abundance of several categories of microepiphytes and total standing crops has been gathered. For Douglas fir, data on the relative distribution of microepiphytes with respect to twig age-class has also been recorded.

Materials and methods

In contrast with the previously published studies on needle microepiphytes cited above, the present investigation was carried out on several trees of four different coniferous species (*Pseudotsuga menziesii* (Mirb.) Franco; *Tsuga heterophylla* (Raf.) Sarg.; *Taxus brevifolia* Nutt.; and *Pinus lambertiana* (Dougl.)) in the hope that extensive sampling might generate more accurate stand-level estimates. All trees sampled were located in watershed 10 of the H. J. Andrews Experimental Forest, an intensive study site for the Coniferous Forest Biome US/IBP. They were growing between 450 and 640 m in elevation in the *Tsuga heterophylla* zone in communities corresponding to the *Tsuga heterophylla – Acer circinatum – Polystichum munitum*, *Pseudotsuga menziesii – Tsuga heterophylla – Castanopsis chrysophylla*, and *Tsuga heterophylla – Rhododendron macrophyllum – Berberis nervosa* types described by Franklin and Dyrness (1973). All trees were sampled between June 15 and August 30, 1972. Site data and descriptive information for each tree are shown in Table 1. A separate publication now in preparation discusses and compares the structure and biomass of these trees in detail. Tree and branch system numbers are provided in Table 1 as a means of allowing data on twig samples to be easily cross-referenced to the more detailed tree descriptions to be published.

Branchlets used for studies of microepiphytes were removed from trees during extensive biomass analyses of both tree and epiphyte components. Branchlets consist of twigs (defined as stems less than 4 cm in diameter) plus attached needles; the procedure by which branchlets were selected for removal and detailed analyses has been described by Pike *et al.* (1977). Branchlets used in the present study were an arbitrarily selected subset of living branchlets; they were taken from overstory trees of *P. menziesii*, *P. lambertiana*, and *T. heterophylla* and understory specimens of *P. menziesii*, *T. brevifolia*, *T. heterophylla*, and *P. lambertiana*. These branchlets are thus expected to reflect reasonably well the variability in twig microepiphyte populations within the watershed.

Microepiphytes were removed from random positions along segments of living twigs. For each branchlet one segment was selected haphazardly from each of nine age-classes. The nine age-classes to be sampled from age-classes 2–15 were selected randomly without replacement and with unequal probabilities

FIELD NUMBER	RATIO: COVER SLIP ZONE AREA / FIELD AREA
10,11	163
9,12	489
8,13	815
7,14	1141
6,15	1467
5,16	1793
4,17	2119
3,18	2445
2,19	2771
1,20	6519

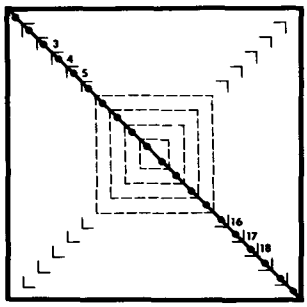

FIG. 1. Scheme for counting microepiphytic cells. Dotted lines and corners within cover slip square denote boundaries of imaginary zones within which microscopic fields were chosen; —, transect across cover slip; ●, one microscope field.

(Raj 1963) proportional to quantities of microepiphytes found in a preliminary survey of tree No. 1109, an old-growth Douglas fir also located on watershed 10. The preliminary survey revealed negligible microbial cell mass on 1-year-old twigs, and consequently this age-class was omitted from the subsequent sample. A single cylindrat (a 3.3-mm-wide belt transect encircling the twig) was delimited at a random position on each segment by turning twigs against two razor blades mounted in parallel 3.3 mm apart in a wooden block. The surface of the cylindrat was moistened with water, and microepiphytes were scraped off using a flattened probe. Care was taken not to dislodge any but a thin layer of bark cells. Microepiphytes were dispersed in a drop of alkaline mounting medium (equal parts of 10% aqueous KOH and glycerine) and completely covered by a 22 mm × 22 mm cover slip.

All fungal and algal cells found in 40 microscope fields at 1000 × were counted and identified by category (see below). Fields were spaced at equal intervals along a transect whose position rotated among vertical, horizontal, and both diagonals of the cover slip. To compensate for the uneven distribution of microorganisms under the cover slip, counts from each field were multiplied by a factor related to the distance of each field from the center of the cover slip (Fig. 1).

Although no attempt was made to distinguish living and dead cells in the samples originally collected for this study, recently collected samples of twig microepiphytes from similar stands have been stained with fluorescein diacetate and observed under the fluorescence microscope (Sörderström 1977). All nonpigmented cells were seen to fluoresce intensely. However, in contrast with Söderström's results, we observed that none of the pigmented fungal cells in the population fluoresced. Although it is possible that such pigmented cells were dead or quiescent, it seems more likely that the fluorescence from the dye was quenched by the wall pigments. Since at least 95% of the twig microepiphytes have heavily pigmented cell walls, no conclusions about relative proportions of living and dead cells are drawn here.

Cell counts were converted to estimates of cell volume by multiplying the estimated number of each taxon by the mean cell volume for that taxon. Cell volumes were determined by measuring diameter on long and short axes of about 100 cells of each taxon. Mean volumes are means of volumes computed separately for each cell measured; the shape of each cell was approximated as a sphere, cylinder, or ellipse rotated about its long axis as deemed appropriate (Table 2). *Aureobasidium pullulans* was found both on the bark surface and inside dead epidermal cells; numbers and cell volumes were computed separately for these two subpopulations and estimates were subsequently added.

Age-specific total cell volume of microepiphytes (*tcv*) was estimated as total cell volume (cubic micrometres per square centimetre) removed from a cylindrat multiplied by the total surface area (square centimetres) for twigs of the corresponding age-class. Twig surface areas were based on measured lengths and diameters of all (or a large subsample of) the twigs in the branchlet. It was intended to estimate total cell volume for all microepiphytes on each branchlet (*tcv*) using ratios based on the selection probabilities (Raj 1963). However, since correlations between sampling probabilities and *tcv* were close to 0, estimates of *tcv* were based on means of *tcv*'s.

TABLE 2. Cell volume estimates of microepiphytes on twigs

Taxon	Shape	Cell volume, (μm^3)	95% confidence interval	*n*
Protococcoid alga	Sphere	189	±27	102
Atichia sp.	Sphere	22	3	151
Metacapnodiaceae (hyphae)	Sphere	758	40	202
Epicoccum (spores)	Sphere	74	13	63
Aureobasidium pullulans, external to bark cells	Cylinder	32	3	114
A. pullulans in epidermal cells	Cylinder	50	7	72
Trentepohlia sp.	Rotated ellipse	3359	611	66

For the purposes of comparing standing crops of various microbial taxa, the categories of cells originally measured and counted (Table 2) were collapsed to four groups which could be unequivocally distinguished: algae; *Atichia*, with a pseudoparenchymatous growth form; Metacapnodiaceae, with trichomelike hyphae; and typical mycelial fungi.

Results and discussion

In discussing twig microepiphytes, differences in microbial population densities and in microbial species composition on different host trees will be treated first. Subsequently, data on the distribution of microepiphytes on Douglas fir twigs with respect to twig age-class will be presented. Finally, stand-level estimates of microbial standing crops based on known tree densities and estimated twig surface

areas for single large Douglas fir trees will be attempted.

Consideration of total estimated microbial population densities on twigs for each of the individual branchlets sampled reveals striking trends with regard to host tree (Fig. 2). Although the estimates for any single branchlet may be highly uncertain, microbial populations on Douglas fir twigs are generally denser than those on *Taxus brevifolia* and *Pinus lambertiana* and more dense than those on two out of the three branchlets sampled from *Tsuga heterophylla*. These differences show no correlation with other environmental variables such as elevation, aspect, or height in the canopy. Although significant effects of environmental variables might well be demonstrated with a more extensive sample, we suspect that the physical and chemical characteristics of the twig bark are of prime importance in regulating populations of twig microepiphytes. Thus, young twigs of pine and yew are smooth and may provide a difficult surface for microbial colonization; in contrast, 1 and 2-year-old twigs of Douglas fir are covered with epidermal hairs, while older twigs are rough and scaly. Such a textured surface may provide more favorable microhabitats for the lodgement and growth of microepiphytic cells.

When the species composition of twig microepiphytes is compared among host trees differences are also seen. Figure 3 shows the absolute amounts of microbial cell volume for each of the four categories of microbial cell recognized. The high uncertainty of the absolute volume estimates renders detailed comparisons pointless. However, several striking patterns emerge which deserve mention: for all host trees, mycelial fungi account for a large portion (35–60%) of the total twig microepiphyte cell volume; the relative cell volume of

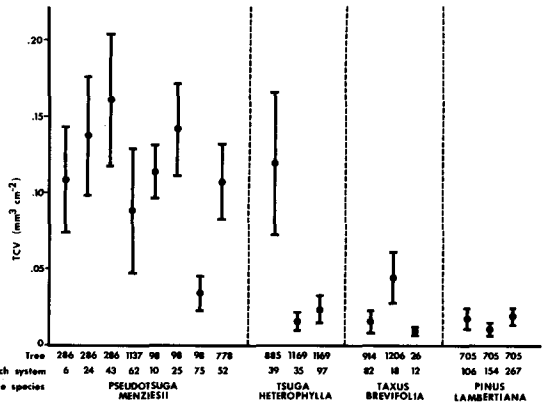

FIG. 2. Estimated microbial standing crops for the various branchlets sampled; *tcv*, total cell volume. Bars represent one standard error above and below the mean.

metacapnodiaceous fungi is high on Douglas fir twigs and lower on twigs of other host trees; algae are a significant component of the total twig microepiphyte population only on *Pinus lambertiana*, largely because of the small absolute volumes of *Atichia* and metacapnodiaceous fungi in that microepiphytic population. The reasons for these apparent microbial preferences for twigs of certain coniferous species are obscure; again they may relate to physical and chemical characteristics of the twig surface.

Consideration of twig microepiphyte distribution with respect to twig age-class has proved possible only for Douglas fir, where the sample size was sufficiently large that all age-classes from 1 to 15 years were sampled at least twice, and certain age-classes were sampled as many as seven times. The data in Fig. 4 were derived by first computing the relative total cell volume for all age-classes sampled on each individual branchlet; in each case the total estimated cell volume for each age-class was divided by the estimated total for the entire branchlet. The relative total cell volumes available for each age-class were then averaged for all branchlets to provide a mean overall relative total cell volume for each age-class.

Although the sample size is too small to reveal differences significant at the 0.05 level between adjacent age-classes, definite trends are evident. Microepiphyte cell volumes are low in relation to available surface area on young twigs. As the twigs age, the microbial populations build up, with the largest total cell volume on 6- and 7-year-old twigs. Thereafter, total cell volumes decline as twig surface area decreases, although microbial population densities appear to remain constant or even increase on older twigs.

Finally, when the estimated total cell volumes per square centimetre for all of the Douglas fir branchlets sampled are averaged, a mean microbial population density for Douglas fir twig surfaces results. If this mean value (0.1113 mm^3/cm^2) is multiplied by the total living twig surface area from 2 to 15 years estimated for tree No. 286 (230.5 m^2), a total microbial cell volume per tree of 257 cm^3 is generated. Microepiphytes on the surfaces of dead twigs have not been considered in the present study. However, they have been observed casually and appear to be at least as abundant as on living twigs. If the population densities are assumed to be the same, and if the 104 m^2 of dead twig surface area estimated for tree No. 286 is considered typical for old-growth Douglas fir trees , an additional 115 cm^3 must be added to the living twig total to yield a tree-level total of 372 cm^3. Data from reference

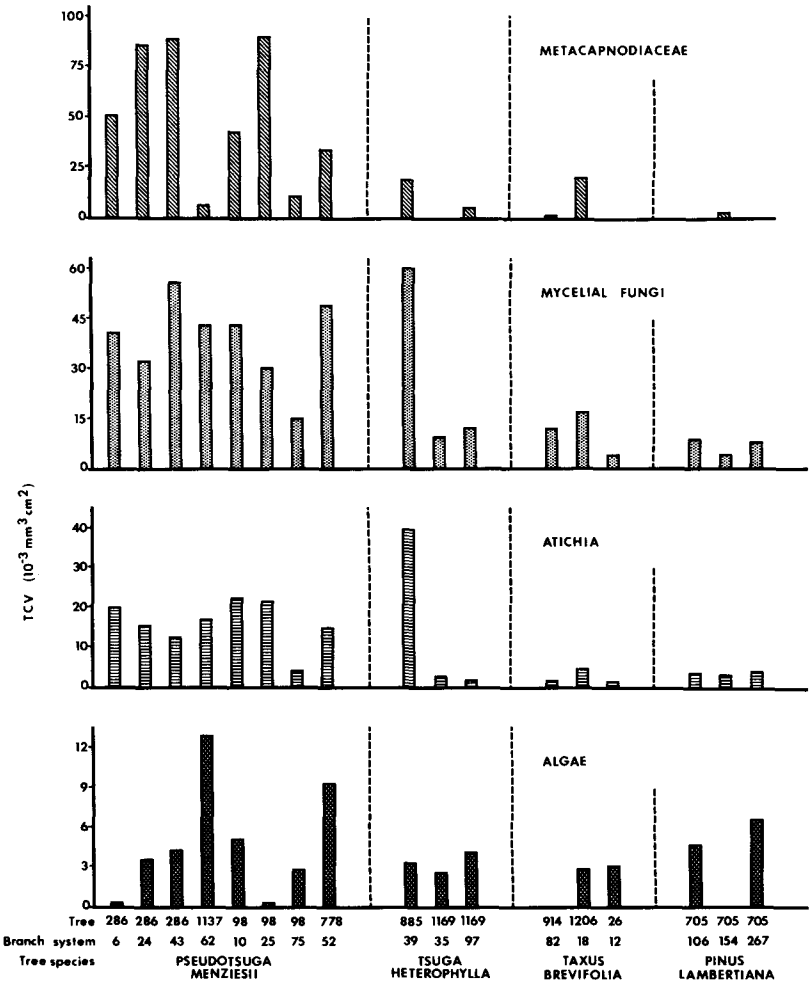

FIG. 3. Estimated standing crops separated by category of microbial cell for the various branchlets; *tcv*, total cell volume. Note that the vertical scale differs for each type of microbial cell.

stands on the Andrews Forest suggest densities of 45 trees ≥ 100 cm diameter at breast height (DBH) for the community types dominant on watershed 10. Information on weights of twigs in understory trees for these communities is not available. However, data from Grier and Logan (1977) suggest that about 10% of the foliage in such stands occurs on understory; if this distribution also holds for twigs, then 50 should prove an appropriate multiplier to expand tree-level estimates to stand-level estimates in these habitats. When the expansion is carried out for twig microepiphytes, an estimate of 18 600 cm^3/ha is generated. If the conversion factor for volume to dry weight for twig microepiphytes is assumed to be about that for needle microepiphytes (0.7–1.1 g/cm^3), this estimate will correspond to a standing crop of 13–21 kg/ha. Fungi account for 95% of the microepiphyte cell volume on Douglas fir twigs (Fig. 3); thus standing crops of fungal microepiphytes on twigs are estimated to fall in the range of 12.4–20 kg/ha. This turns out to be significant in comparison with the 30 kg/ha of fungal biomass projected for needle surfaces (Carroll 1979). Thus, twig microepiphytes must be considered in any investigation on the role of epiphytic fungi in nutrient cycling in temperate coniferous forests.

On the basis of data on the relative production of fungi in several habitats and of algae epiphytic on larch twigs, Carroll (1979) suggested that the relative annual production of microorganisms on Douglas fir needles might be as high as 1000%, a level corresponding to the assimilation of 600 kg of organic matter/ha per year. If similar assumptions

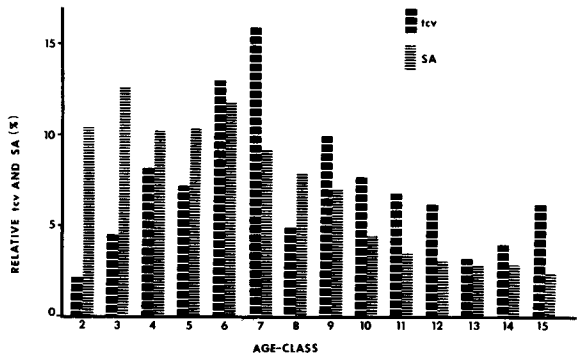

FIG. 4. Relative distribution of microbial cell volumes by twig age-class for *Pseudotsuga menziesii*; *tcv*, total cell volume per age-class; SA, surface area; (%), percent of total *tcv* and SA estimated for the 14 age-classes.

are made for twig microepiphytes, and a figure of 15 kg/ha fungal standing crop is accepted, twig microepiphytes might account for the assimilation of an additional 300 kg/ha per year in primary production. The total secondary microbial production for microepiphytes on needles and twigs may thus range up to 450 kg/ha per year, corresponding to 900 kg/ha per year in primary production (assuming 50% conversion rate) for old-growth Douglas fir stands. While these figures are, at best, guesses, entirely unsupported by data on growth and respiration rates of twig microepiphyte populations, they appear reasonable in light of recent studies by Flanagan and Bunnell (1976). On the basis of measurements of fungal standing crops in the field and of growth and respiration rates in the laboratory these authors have estimated annual production of fungi inhabiting dead leaves of *Eriophorum* in a tundra habitat. Their estimates suggest an annual production of 15 generations of the microbial mean standing crop or a relative annual production of 1500%.

Since fungal microepiphytes in the canopy are largely superficial, fixed carbon derived from the decomposition of canopy substrates probably represents only a minor portion of that necessary to support the annual production suggested above. The metacapnodiaceous fungi (MCAP), a dominant group of twig microepiphytes, are widely reported to subsist on the excreta or honeydew from homopteran insects (Reynolds 1975). Since such insects comprise a minor portion of the fauna in the old-growth Douglas fir stand studied here, alternate sources of carbon must be utilized by these fungi. Recent work by Cooper and Carroll (1979) has revealed the presence of ribitol as a major component of leachates from *Lobaria oregana* (Tuck.) Müll Arg., the dominant epiphytic lichen in the canopies under study. Ribitol has also been reported as a major constituent of scale honeydew (Fraser 1937; Hackmann and Trikojus 1952); Fraser (1937) showed it to be a suitable carbon source for the *in vitro* culture of many epiphytic sooty molds. Consequently, fungal microepiphytes in this canopy probably have available to them an appropriate carbon source for growth in the form of organics leached from other canopy components during rainy periods (from September through May in the Pacific Northwest). We have yet to determine whether the quantity of such leachates would suffice for the levels of microbial production estimated above. Experiments designed to simulate canopy leaching in the laboratory are currently in progress here with a view towards answering this question.

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