Needle microepiphytes in a Douglas fir canopy: biomass and distribution patterns

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Distribution patterns and total cell-volume estimates for needle microepiphytes are presented for three strata in the canopy of a single old-growth Douglas fir tree. Microbial cell volume was estimated by photographing transverse sections of needles, tracing microbial profiles on Mylar film, cutting out the tracings, and determining the pooled trace weights from various zones of each needle section. Microbial cells are concentrated in the midrib groove and over the stomatal zones of individual needles. Microbial cell volume on the upper needle surfaces increases during the 1st year and declines in subsequent years. Cell volumes on the lower needle surfaces increase from the 1st to the 3rd year and decrease from the 3rd to the 4th year. An increase in microbial cell volume occurs on both upper and lower surfaces from year 7 to year 8. Total microbial cell volume in relation to available needle surface area is greatest in the lower canopy and decreases with increasing height in the canopy. The total volume of microbial cells on needles was estimated to be 1093 cm^3 for the entire tree.

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Des types de distribution et l'estimation du volume cellulaire total des microépiphytes des aiguilles sont présentés pour trois strates différentes au sein de la couronne d'un seul sapin Douglas âgé. Le volume cellulaire microbien a été estimé en photographiant des coupes transversales d'aiguilles, en décalquant les contours des colonies microbiennes sur film Mylar, en découpant ces images et en déterminant les poids de l'ensemble des images provenant des diverses zones de chaque coupe d'aiguille. Les cellules microbiennes sont concentrées dans le sillon formé par la nervure médiane et sur les rangs de stomates de chaque aiguille. Le volume cellulaire microbien sur la surface supérieure de l'aiguille augmente au cours de la 1ère année d'âge et diminue au cours des années suivantes. Le volume cellulaire sur la surface inférieure de l'aiguille augmente chez les aiguilles âgées de 1 à 3 ans et diminue chez les aiguilles âgées de 3 à 4 ans. Un accroissement du volume cellulaire microbien s'observe à la fois sur les surfaces supérieure et inférieure des aiguilles âgées de 7 et 8 ans. Le rapport entre le volume cellulaire microbien total et la surface d'aiguille disponible est maximal dans la partie inférieure de la couronne de l'arbre et décroit avec la hauteur dans la couronne. Le volume total de cellules microbiennes sur les aiguilles a été estimé à 1093 cm^3 pour l'arbre entier.

Introduction

The existence of diverse and abundant microbial populations on external aerial plant surfaces has by now been well documented (Preece and Dickinson 1971; Dickinson and Preece 1976). The majority of studies on phyllosphere microorganisms have, however, relied on frequency data or plate counts and have focused on interactions within restricted microbial communities. Quantitative data on standing crops and annual production, which might be used to assess the role of phyllosphere microorganisms in elemental cycling at an ecosystem level, are almost nonexistent. Where such data are available (Diem 1974; Bernstein and Carroll 1977) they are expressed in terms of microbial cover, a measure which cannot be converted to standing crops without additional information on the thickness of microbial colonies and the appropriate cell volume – dry weight conversion factors. The present study was undertaken to corroborate and further define microbial distribution patterns previously reported for needles of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) by Bernstein and Carroll (1977) and to estimate microbial standing crops on needle surfaces as a first step towards estimating annual production.

Materials and Methods

Sample Collection

Foliage was taken from four heights (33, 53, 61, and 68 m) in the canopy of a single large (77 m tall) Douglas fir tree (El Capitan) in September 1976. Samples were collected by tree climbers using methods of ascent previously described by Denison *et al.* (1972). El Capitan is located in the H. J. Andrews Experimental Forest (44°10′ N latitude, 122°20′ W longitude) in the western Cascade Mountains approximately 70 km east of Eugene, Oregon. The tree occurs in a stand corresponding to the *Tsuga heterophylla – Rhododendron macrophyllum – Berberis nervosa* community of the *Tsuga heterophylla* zone (Franklin and Dyrness 1973).

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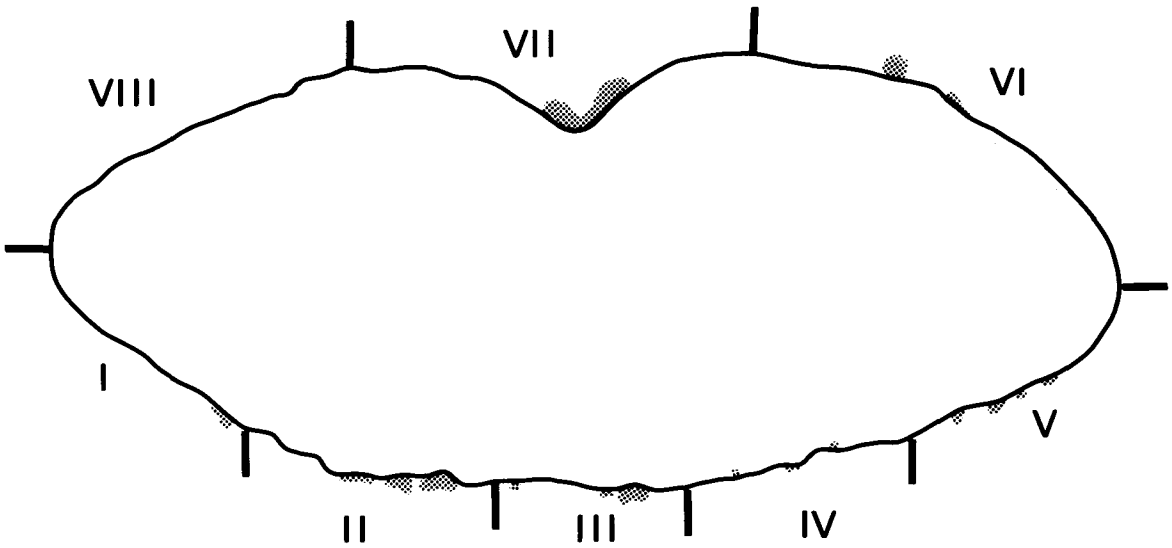

FIG. 1. Profile of median cross section from a typical Douglas fir needle. Zones, indicated with Roman numerals, are defined in the text. Stippled areas on the surface represent profiles of microbial cells. Small stippled areas on the inner face of the needle profile represent algal cells within front cavities of stomata.

Laboratory Measurements

Five twig segments from each foliage sample were arbitrarily selected from age-classes 1–8 years; a single needle was chosen from each twig segment using a random-numbers table, giving a sample of five needles for each of eight age-classes at four different heights in the canopy. The length of each needle was measured to the nearest 0.5 mm. A random position was marked on each needle and a series of four to eight transverse free-hand sections was cut with a razor blade within a 1-mm zone symetrically placed about that position. Sections were mounted on microscope slides in lactophenol with 0.5% aniline blue, and the slides were gently heated to remove air bubbles from the sections. Three individual sections per slide were chosen arbitrarily on the basis of thinness and integrity and were circled for subsequent photography. Photographs were taken on Kodak Panatomic X film with a Zeiss Universal photomicroscope equipped with an automatic camera. Photographs of needle sections were printed on Kodabromide III paper to a final magnification of 228×. Tracings of needle perimeters were made on sheets of tracing paper laid over the original photographs, and cross-sectional profiles of epiphytic microbial cell mass were marked in on the tracing in red pencil by reference to the original section viewed under the compound microscope. On each tracing the needle perimeter was divided into zones as follows: I and V, lower shoulders; II and IV, stomatal zones; III, midrib; VI and VIII, upper shoulders; VII, midrib groove. Thus zones I–V comprised the lower surface of the needle and zones VI–VIII, the upper surface (Fig. 1; see also Bernstein and Carroll 1977 for a more detailed description of needle morphology). Cross-sectional profiles of microbial cell mass were traced onto Mylar film, cut out with a stencil knife, and attached onto a labelled board with insect pins (Fig. 2). Mylar cutouts were weighed on a microbalance to the nearest microgram and weights were recorded for the pooled cutouts from each zone of each needle section. Weights (in milligrams) were converted to actual microbial cross-sectional areas (in square micrometres) by multiplying by 161.6, a constant which incorporates both the density of the Mylar film (0.1185 mg/mm^2) and the magnification of the photographs (× 228). Perimeters for each zone of each needle section and perimeters overlain by microbial cell profiles within each zone were determined with a map measurer.

Data Reduction and Statistical Analysis

In the course of the study, four sets of variables were measured directly: needle length, microbial cross-sectional areas, needle and zone perimeters, and projected microbial perimeters (that portion of the needle perimeter overlain by microbial cell mass). Three other variables were derived from these primary measurements: mean microbial cell volume per needle (needle length × microbial cross-sectional area), percent cover (projected microbial perimeter per zone or needle perimeter), and microbial cell thickness (microbial cross-sectional area per projected microbial perimeter). For all measured variables the mean of the values for the three sections was taken as an estimate of the true value for that needle. Error statistics for the derived variables were computed on the basis of five such means from the five independently chosen needles in each category (age-class and height in canopy); the specifics of the computation differed depending on the form of the derived variables. Since microbial cell volumes per needle were derived as products, the means and standard errors were determined by conventional statistical methods. Percent cover and microbial cell thickness were derived in the form of ratios; consequently, for these variables, the ratio of the means was taken as the least biased estimate of the population mean (Kendall and Stuart 1966) and standard errors were computed as described by Raj (1963). Although error statistics are not shown on the following graphs, standard errors were generally 25–50% of the mean values. Since the data were highly heteroscedastic, a nonparametric procedure, a two-tailed Mann–Whitney–Wilcoxon test was used to assess the significance of differences of estimates of population means in pairwise comparisons (Gibbons 1976).

In discussing patterns of microbial cell distribution both total microbial cell volume per needle and percent cover were computed for all four heights in the canopy and for all eight age-

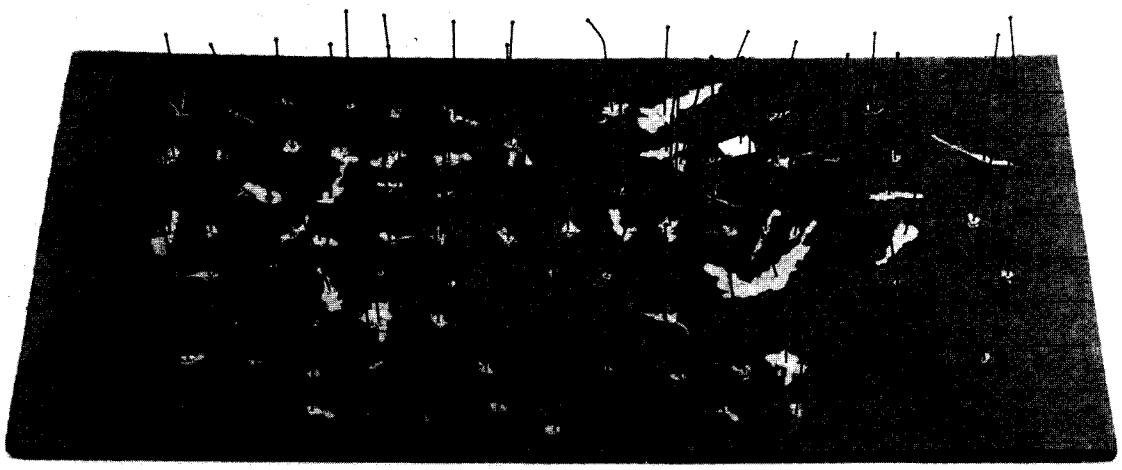

FIG. 2. Labelled pinning board with cut-out tracings of microbial cell profiles.

classes for which data were available. In computing tree-level totals and proportional distributions over the entire tree, only three canopy strata and seven age-classes were considered. Boundaries for canopy strata were defined according to height (metres) from the base of the tree as follows: lower canopy, 15–36 m; middle canopy, 36–56 m; upper canopy, 56–77 m. Mean microbial cell volumes per needle for samples from 61 and 68 m were averaged for each needle class to obtain a mean value for the upper canopy. So few needles of age-class 8 were present that their numbers and surface areas rounded to zero in the existing tree-level expansions of El Capitan. Numbers of needles and needle surface areas by age-class and canopy stratum were estimated on the basis of tree descriptions and sampling conducted previously using methods detailed by Pike *et al.* (1977). The proportion of total needle surface area in each of the needle zones designated above was taken as the average zone perimeter per needle perimeter ratio for the entire set of needles after ANOVA did not show any systematic variation of these proportions with needle age or canopy stratum. Total microbial cell volumes per stratum were estimated by multiplying the mean microbial cell volume per needle for a stratum by the total number of needles in that stratum.

Results and Discussion

Distribution Patterns

In considering microbial distribution patterns on needle surfaces, microbial cell volume (*MCV*) has been considered the most useful and direct estimator of biomass and abundance. Data on other derived variables such as percent cover and microbial cell thickness are presented only for the purposes of comparison with similar data in the literature or when they seem to provide additional information. Patterns of nonrandom occurrence are apparent on several scales within the canopy: over the surface of individual needles, over a series of needle age-classes within individual branch systems, and from one canopy stratum to the next within the entire tree. Additional stand-level patterns from one tree to the next doubtless exist, but these have not been examined in the present study. Microbial distribution patterns are described below in order of increasing scale.

Zonal Distribution

When the distribution of microbial cell volume is examined with respect to position on individual needles, distinct patterns emerge (Fig. 3). Microbial cells are concentrated over the stomata (zones II and IV) and in the midrib groove (zone VII). Collectively, these zones account for more than 70% of the total *MCV* in the canopy although they occupy only about 35% of the total needle surface area; the upper and lower needle shoulders (zones I, V, VI, and VIII) and the midrib (zone III) are correspondingly depauperate of microbial cells. Upper and lower needle surfaces also show consistent differences in *MCV* which are generally significant at $P < 0.1$ level and are often significant at the $P < 0.05$ level; *MCV* estimates are greater on the bottom of the needle than on the top, except for 1- and 2-year-old needles (Figs. 3–5). Initial colonization of the needle surfaces appears to proceed faster on the top than on the bottom. Microbial cover shows patterns similar to *MCV* (Figs. 4, 5). No significant differences in microbial cell thickness can be shown between top and bottom surfaces for any individual class of needles. However, when all age-classes of needles from all strata are considered, the sign test demonstrates that cells are significantly ($P < 0.01$) thicker on the upper surface.

Bernstein and Carroll (1977) have noted similar significant ($P < 0.05$) differences in microbial cover between upper and lower surfaces of Douglas fir needles from another old-growth tree from a stand adjacent to the Andrews Forest. They suggest that a relatively shallow topography and exposure to

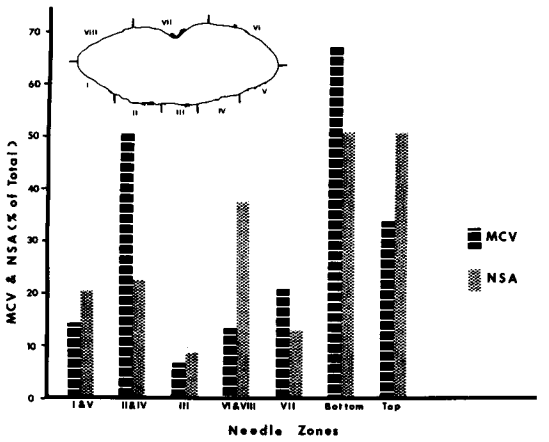

FIG. 3. Relative distribution of total microbial cell volume (*MCV*) and total needle surface area (*NSA*) by needle zone.

extremes of microclimate on the upper needle surfaces account for these differences. The more detailed description of microbial abundance presented here reinforces these conclusions. Microbial cells are concentrated in protected zones of high relief on the needle surface (the mid-rib groove and stomatal zones). In fact, much of the microbial standing crop on the lower needle surface consists of algal cells which occur actually within the front cavities of the stomata themselves.

Age-class Distribution Patterns

The abundance of microbial cells on needle surfaces varies with regard to needle age. Although a small sample size ($n = 5$) has seldom allowed differences in *MCV* significant at $P < 0.05$ to be demonstrated, consistent trends are evident in the four branch systems sampled (Figs. 4, 5). Microbial cell volumes on the upper needle surfaces generally increase between age-classes 1 and 2 and fall between age-classes 2 and 3 (or between age-classes 3 and 4 in the sample from 68 m), while on the lower needle surfaces *MCV* increases until age-class 3 and falls between age-classes 3 and 4. For almost all needle surfaces an increase in *MCV* on 8-year-old needles is evident. In the upper canopy (61 and 68 m) an intermediate peak appears at 5 years, however, its existence must be considered rather uncertain in the absence of sufficient replication.

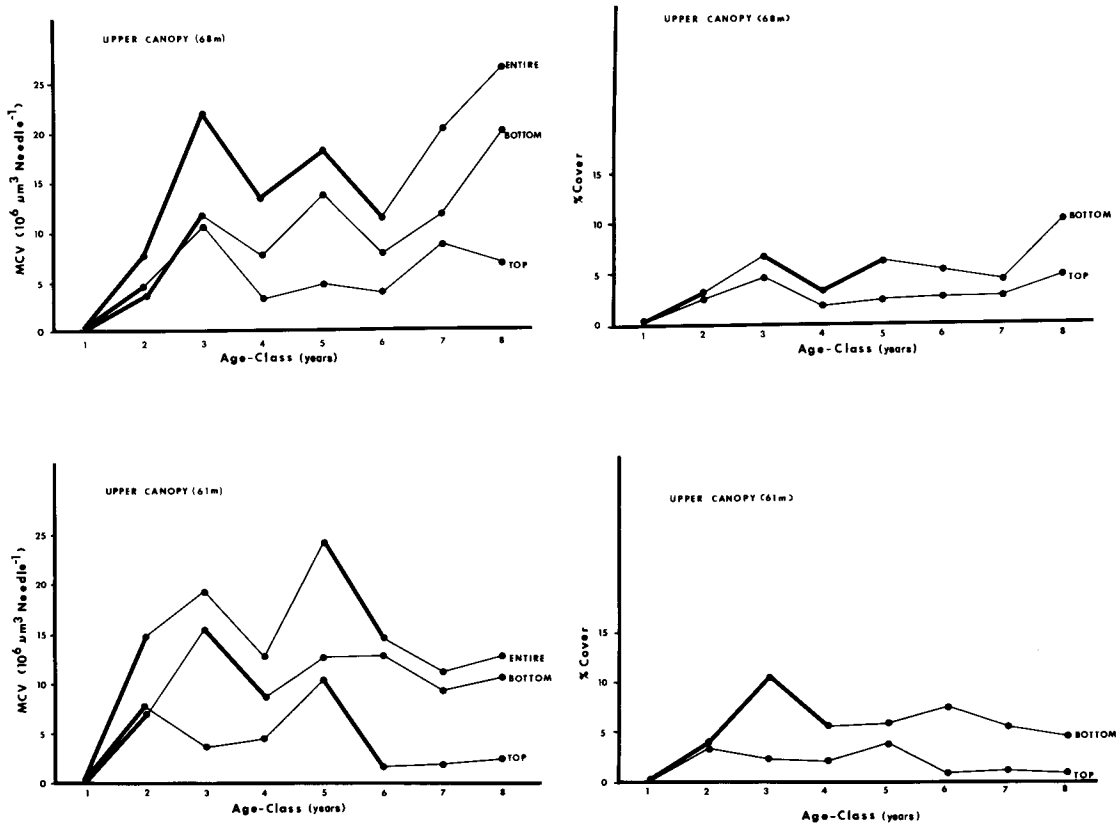

FIG. 4. Change in microbial cell volume (*MCV*) per needle and percent cover with needle age at several heights in the canopy. Needles produced in the current year, beginning with bud burst in June, are considered to belong to age-class 1. Thicker lines indicate differences in the estimates of population means for adjacent age-classes significant at $P < 0.1$ level.

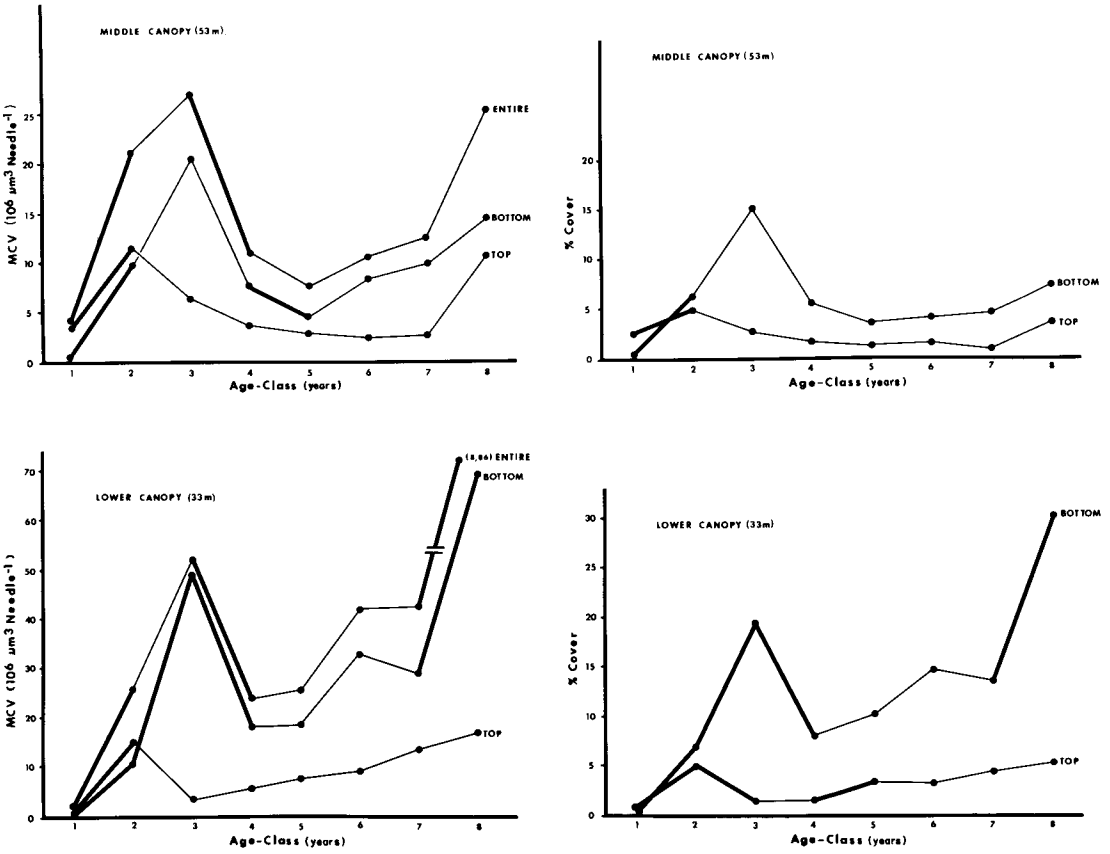

FIG. 5. Change in microbial cell volume (*MCV*) per needle and percent cover with needle age at several heights in the canopy. Needles produced in the current year, beginning with bud burst in June, are considered to belong to age-class 1. Thicker lines indicate differences in the estimates of population means for adjacent age-classes significant at $P < 0.1$ level.

When the distribution of microbial cell volume by age-class is computed for the entire tree, 1-year needles are found to have little *MCV* in relation to the surface area available for colonization, while 3-year needles develop much more in relation to their total surface area (Fig. 6). In fact, over 40% of the total *MCV* on the tree is found on 3-year needles. A cautionary note must be appended to these data, however. Perusal of Fig. 6 reveals that needle surface area increases from year 1 to year 3 before dropping off at year 4 and older age-classes. This pattern has been found in samples collected in several different years from a number of old-growth trees in two different sites. With Douglas fir, the number of needles produced in a given year is determinate in that the needle primordia have developed during dormant bud formation the previous summer; no new needles are formed on a given twig segment in years subsequent to that in which the segment itself was produced (Allen and Owens 1972). Consequently, the distribution of needle surface area described above is impossible on a sustained basis. Pike *et al.* (1977) have attributed this spurious distribution to their inability to detect dormant bud burst and resultant missing age-classes along a twig axis; as a result, the biomass and surface area of 2- and 3-year needles is overestimated and that of older needles is underestimated. If such is assumed to be the situation for El Capitan, a portion of the surface area and a corresponding

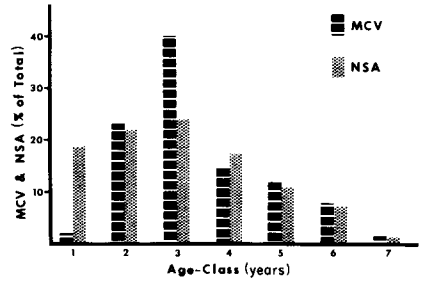

FIG. 6. Relative distribution of total microbial cell volume (*MCV*) and total needle surface area (*NSA*) by needle age-class. Needles produced in the current year are considered to belong to age-class 1.

proportion of the *MCV* for years 2 and 3 should be spread over years 4–7. While this will alter the pattern shown in Fig. 6 slightly, it may affect computation of total *MCV* for the entire tree (see below).

Patterns of microbial cover with respect to needle age have been plotted to assess the usefulness of percent cover as an estimator of standing crop and to provide a basis of comparing results presented here with those of Bernstein and Carroll (1977). Reference to Figs. 3 and 4 reveals that percent cover reflects the distribution patterns of *MCV* by age-class with great fidelity. Without exception, every trend significant at the $P < 0.1$ level for *MCV* also appears in the plots for percent cover. Comparison of percent cover data from the present study with those of Bernstein and Carroll (1977) reveals an amazing degree of congruence in certain cases, particularly in view of differences in the methods used. Bernstein and Carroll observed needle surfaces directly under the fluorescence microscope with epiillumination and estimated cover by visually assigning quadrats in a belt transect to cover classes (percent cover in the present study has been determined by direct measurement). Similarities in patterns of cover are particularly evident in the lower canopy of tree 286 and El Capitan, the similarities become even more striking when years 4 and 6 are omitted in constructing the plots for El Capitan as they were in the previous study on tree 286 (compare Fig. 4 (Bernstein and Carroll 1977) with Fig. 5, lower canopy, in the present study). Patterns of microbial cover with respect to needle age-class are much less similar for middle and upper canopy in the two studies. This discrepancy may reflect real differences in the trees studied. Alternatively, examination of all age classes in the previous study might well have generated patterns more similar to those seen here; the consistent decline in percent cover observed on 4-year-old needles in the present study would have been missed by Bernstein and Carroll (1977) since age 4 needles were not examined.

Several explanations can be advanced for the observed changes in *MCV* and microbial cover with respect to needle age-class. Three-year-old needles with a heavy load of microepiphytes may be subject to selective early abscission; further buildup and abscission with older needles could account for intermediate peaks in *MCV* per needle evident in the upper canopy. A similar explanation has been suggested by Sherwood and Carroll (1974) to explain high incidences of infection by the ascomycete *Schizothyrium* on young needles and low infection rates on 7- and 8-year needles. In addition, selective grazing by microarthropods may account for some of the pattern observed here. Populations of microarthropods in the Douglas fir canopy have been sampled intensively in this laboratory by Dr. David Voegtlin. Numerous species of mites have been observed on needle and twig surfaces. Preliminary evidence suggests that they partition the habitat on a fine scale. Thus, a species of *Camissia* commonly occurs only on surfaces of 4-year-old twigs; this mite has been observed to feed on microbial cells from the twigs as well as on cultures of several of the common fungi from twig surfaces (André and Voegtlin, unpublished). Other mites, as yet less well investigated, may feed on microbial cell mass from selected age-classes of needles, thus accounting for the observed changes in *MCV* from one needle age-class to the next.

Stratum-level Distribution Patterns

Comparison of mean microbial cell volumes for various age-classes at the stratum level reveals large amounts of microbial cell volume per needle in the lower canopy and less in the middle and upper canopy (Figs. 5, 6). These differences are significant at the $P < 0.05$ level for needle ages 3–8 years and at the $P < 0.1$ level for 2-year needles (*MCV* per needle for 1-year needles are so low as to render comparisons meaningless). No significant differences in *MCV* per needle are seen between the middle and upper canopy. When total estimates of *MCV* and needle surface area are computed for each of the strata and compared, a progressive decrease in the total *MCV* per stratum in relation to total needle surface area per stratum is seen from the lower canopy to the upper canopy (Fig. 7). Thus, while the lower canopy has 30% of the total *MCV* in the tree, it has less than 20% of the total surface area; the middle and upper canopy have less *MCV* per unit needle surface area. This trend can be shown graphically for both top and bottom needle surfaces as percent total *MCV* minus total needle surface area for the three canopy strata (Fig. 7, inset).

Bernstein and Carroll (1977) were unable to show any significant changes in microbial cover with height in the canopy. This probably relates to the relative insensitivity of cover data as an estimator of microbial standing crops and the impossibility of calculating stratum-level total microbial cell volumes on the basis of such data alone. Reynolds (1972) conducted a cursory study on the stratification of epiphylls in the canopy of a Costa Rican rain forest. His data suggest the selective occurrence of epiphyllous fungi and algae in the lower canopy (lower 10 m). He suggests higher available moisture

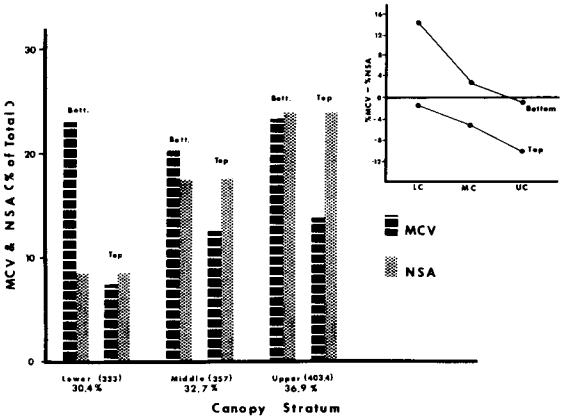

FIG. 7. Relative distribution of total microbial cell volume (*MCV*) and total needle surface area (*NSA*) by canopy stratum and needle surface (top versus bottom). Numbers in parentheses give total *MCV* (cubic centimetres) for the stratum; percentages below refer to the precent of total *MCV* in a given stratum. Bott., bottom; LC, lower canopy; MC, middle canopy; UC, upper canopy.

and lower levels of radiation in the lower canopy as the major factors in determining this distribution. While these must certainly affect the distribution of microepiphytes in the canopy of temperate zone rain forests as well, unpublished results from this laboratory suggest additional significant influences. Populations of mycophagous mites are known to be higher in the upper canopy of the trees we have investigated (D. Voegtlin, unpublished); more intense grazing pressure may reduce standing crops of microbial cell mass. Studies on the leaching of soluble organic components in the laboratory have shown that more polyols and organic nitrogenous compounds are lost from the nitrogen-fixing lichen *Lobaria oregana* (Tuck.) Müll Arg. collected from the lower canopy than from *Lobaria* collected from the upper canopy during simulated rainstorms (Horstmann *et al.* unpublished). Further, the lower canopy will intercept canopy wash from upper strata which has been already enriched in soluble organics. Thus, microepiphytes on needles in the lower canopy can be presumed to exist in an environment richer in dissolved organic nutrients than similar organisms in the upper canopy.

Tree and Stand-level Estimation of Microbial Standing Crops

When the mean *MCV* per needle for each stratum and age-class is multiplied by the total number of needles estimated to fall in each age-class for a given stratum, an estimate for the total *MCV* for the stratum is generated. A total for the entire tree can be derived simply by summing the estimates for each canopy stratum. When this is done, the following values result: lower canopy, 333 cm^3; middle canopy, 357 cm^3; upper canopy, 403 cm^3; total for entire tree, 1093 cm^3.

While error statistics have not been extended to these tree-level estimates, some discussion of uncertainty and bias in the final number may be in order. A systematic error in estimating the proportion of foliage in each needle age-class has been noted above. The net effect of this bias on the tree-level estimates of *MCV* may be slight; however, the decrease in year 3 surface area, with its large amounts of *MCV*, should be balanced by increases in year 8 (and in the upper canopy, year 5) surface area with comparably high levels of microbial cell mass. Standard errors for *MCV* for each needle age-class are large (25–50% of the mean); when these are multiplied by uncertainty in the estimates of needle numbers in each age-class and stratum, even larger standard errors for tree-level estimates will result. Several considerations mitigate this potentially large uncertainty. The large standard errors are in part the result of a small sample size. No significant differences in *MCV* per needle could be demonstrated for the three samples from middle and upper canopy strata. If these were lumped by age-class, a sample size of 15 instead of 5 would result, and the standard errors would be reduced correspondingly. Beyond this, the tree-level estimates presented here have been derived by taking the sum of the means of a number of subsamples of the population; the cancellation of error and reduction of uncertainty in the summation process may be considered a corollary of the law of large numbers.

If the total needle *MCV* estimated for El Capitan is projected to the stand level, an order-of-magnitude approximation for microbial standing crops on needles in old-growth Douglas fir forest can be generated. In terms of needle biomass, El Capitan has proved typical of large (> 100-cm diameter at breast height (dbh)) trees in such old-growth stands (Pike *et al.* unpublished); El Capitan is estimated to have approximately 200 kg of foliage. Data from reference stands on the Andrews Forest suggest densities of 45 trees of 100-cm dbh per hectare for this community type. Data from Grier and Logan (1977) show an additional 1000 kg of foliage in smaller, understory trees for the same community type; this corresponds to the weight of foliage in five trees of the stature of El Capitan. Thus, at the stand level, one might expect total *MCV* per hectare to be on the order of 50 times that estimated for El Capitan (a value of 55 000 cm^3/ha). Factors for the conversion of volume to dry weight have not been critically determined for needle microepiphytes; however, dry weight to fresh weight

ratios have been measured for mechanically detached needle microepiphytes and for isolated cells from pure cultures of *Aureobasidium*, *Atichia*, and *Cladosporium* from needle surfaces. These lie in the range of 0.6–0.9 g/g, much higher than the 0.1 g/g normally assumed for plant cells. If the density of dry cells is assumed to be 1.2 g/cm^3, a conversion factor of 0.7–1.1 g/cm^3 may be appropriate. This would yield a biomass of 38–60 kg for the estimated volume of 55 000 cm^3.

Data on relative production which might be used to project annual production from standing crop are totally lacking. However, several values for similar microorganisms are available from the literature. Relative annual production of soil fungi may be at least 1000% (Nagel-de Boois and Jensen 1971). Data from Waid *et al.* (1973) suggest relative production of active mycelium on the surface of recently fallen leaves of 900% in the interval of 2 weeks. The data of Turner (1975) coupled with an assumed algal chlorophyll content of 1–2% yield annual relative production estimates of 180–720% for *Pleurococcus* growing on twig surfaces in larch plantations in Britain. It seems possible that annual relative production for the microbial populations described in the present study may be as much as 1000%. Much of the standing crop estimated here, perhaps 40%, is composed of algal cells. If the remaining 60% of the observed cell volume is fungal, it might correspond to perhaps 30 kg of biomass. Assuming 1000% relative production and a 50% conversion rate of assimilated organics from canopy leachates (Calow 1977), this population might account for 600 kg/ha per year in primary production which would be missed in conventional carbon-budget accounting procedures for ecosystems analysis.

Much of the above argument rests on speculation and reported values of microbial production for rather dissimilar habitats. Experimental determination of relative microbial production on needles will be essential if annual production is to be estimated from standing crops with any precision. This will require the development of simplified spectrophotometric or gravimetric techniques which can be used to estimate standing crop on many samples at frequent intervals. Such procedures are currently being actively explored in this laboratory.

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