INTERNAL REPORT 114

MICROBIAL POPULATIONS ON CONIFEROUS FOLIAGE AND TWIGS

Progress Report

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Coniferous foliage and green twigs comprise a major portion of the total photosynthetic surface within the coniferous biome. Insofar as the net primary productivity of the biome depends on the efficient functioning of this surface, microorganisms living within or on needles and twigs may exert a disproportionately large influence on the overall activities of the forest. Obvious effects include the direct utilization of carbohydrate produced by the needles and hastened senescence of the needles through infection. More subtle effects may involve increases in the leaching of soluble carbohydrates from the photosynthetic surfaces as a result of microbial erosion of the leaf cuticle.

During the 1972 calendar year (Biome year 2) we have attempted to begin making crude estimates of the size of the microbial population in the forest canopy (in kg/ha) and the rate at which this population turns over. The methodology for estimating microbial standing crops in the canopy is now well developed, and much of the necessary data has been collected and is awaiting computer processing at Oregon State University. A preliminary survey of the microbial populations of needles and twigs revealed that most of the organisms are entirely superficial and that their relative abundance depends very much on the age of the substrate. Thus it became clear that even crude estimates of standing crop must take into account the absolute surface areas of needles and twigs in the canopy by age class. In conjunction with the structure and biomass program conducted by investigators

(Denison, Overton, Pike, Lavender, and others) at OSU, sample branch systems were cut from computer selected old growth trees and the portions under 4 cm in diameter were brought into our laboratory for further processing. This material was cut up by year's growth into 15 age classes and a 15 yr.-4 cm category. Sacs of samples were dried, and twigs and needles were separated and weighed. The twigs have been measured and surface areas calculated by age class. Measurement of the needles is still in progress, but should be completed within several months. Relationships between dry weights of needles and twigs of a given age class and their surface areas will be calculated in the near future. The data will then be extrapolated to the entire trees and ultimately to the entire watershed (H. J. Andrews Watershed #10) on the basis of computer assisted sampling conducted last summer.

Estimation of microbial biomass for the various age classes of needles and twigs is being carried out by visual inspection through the microscope. For the twigs 1 cm wide, randomly chosen "cylindrats" of bark are stripped and soaked in KOH. After rinsing, the mats of mycelium with entrapped algae, yeast, bacteria, and insect excreta (collectively known as "scuzz") are scraped off and mounted for microscopic examination. Estimates of total microbial cell volume on the slide can be made on the basis of cell counts along random transects across the mount. Volume to wet weight conversions are based on average figures for microbial cell density. Wet weight to dry weight conversions can be guessed at by comparison of wet and dry weights of pure culbures of several of the most frequently encountered fungi. Estimates of microbial populations on needle surfaces are being carried out by visual examination of primulin-stained needles under a fluorescence microscope equipped with epi-illumination. Volume to dry

weight conversions will be carried out as with the twigs. Although estimates of microbial standing crops in the canopy are of course dependent on total surface area estimates, which are far from completion, preliminary calculations suggest totals in the range of 10-100 kg/ha dry weight during the summertime.

Rates of microbial turnover in the canopy do not lend themselves to the direct methods of measurement employed for standing crop estimates. Export of microbial cells may take place in the throughfall during the winter, to the air via aerial spores during the summer, and conceivably through <u>in situ</u> grazing by small animals at any time. We are now in the process of estimating the extent of cell export in th throughfall. We do not anticipate attempting to determine the extent of spore fall during the spring and summer although this needs to be done. I see no obvious way of estimating the amount of microbial cell biomass consumed by small animals.

Samples of rainwater are being collected at various levels in the canopy in specially designed collectors made from high temperature polypropylene funnels and bottles. Sterile bottles are inserted in the holders during a rainstorm, and a series of holders is holsted into the canopy on a rope attached to a hanger left in the tree from last summer's climbing operation. After several hours the samplers are lowered to the ground, and the bottles containing rain water are placed on ice. The samples are then immediately taken back to Eugene where they are run through GE Nucleopore microbiological filters which have been previously tared. The filters and adhering microbes are then dried and reweighed, and the dry weight of the microbial cell mass is calculated. Visual examination of the filters under the microscope suggests that about 50% of the material suspended in the

throughfall consists of small pieces of bark, beetle frass, and bits of inorganic matter; the other half consists of microbial cells. Preliminary results suggest minimum values of 500 kg/ha/yr for the total microliter we are measuring. This may well correspond to 10 kg of fixed nitrogen returned from the canopy sub-system to the forest to the forest floor. Thus in any computer model of the system, microlitter must be considered a significant component.

In the coming year the above sorts of measurements will be extended and refined. The project is being terminated at the end of 1973, and we do not anticipate delving into perhaps more fundamental questions such as the role of canopy microbes in needle senescence or their possible function in decomposing already inoculated litter once it hits the forest floor.

Three research assistants have figured prominently in the development of techniques and in carrying out the actual measurements. These are Martha Sherwood, Mary Bernstein, and John Perkins. They will of course author or co-author papers describing the work in which they have been involved.

