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NITROGEN FIXATION (C_2H_2 REDUCTION) BY LUNGWORT LICHEN (LOBARIA PULMONARIA (L.) HOFFM.) ON RED MAPLE (ACER RUBRUM L.)

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A study of nitrogen fixation using the acetylene reduction technique by the lungwort lichen was carried out to investigate the nitrogen inputs of a forest stand. The lichen grew predominantly on red maple, occurring on 23% of the stems and reaching the highest coverage on trees of DBH 20 to 25 cm. Total coverage of the lichen was 12.7 m^2 ha^{-1}.

Forty-seven acetylene reduction assays indicated that N_2 fixation rates were very variable principally as a result of variation in thallus moisture. Cumulative N_2 fixation for each month was estimated from precipitation, temperature, and day length data. Stemflow was also collected and samples from above and below the lichen were compared.

Soils in the study area had only 2 kg ha^{-1} of nitrate so even small inputs are important. However, the total input from lungwort was calculated to be only 3.5 gN ha^{-1}. The maximum rate estimated for a single tree would be equivalent to about 5% of the annual nitrogen requirements of the canopy foliage. Although these rates are about three times those of others reported in the literature, they are not believed to be very important.

The stemflow studies also indicated that this input was small, and decomposition of the lichen thallus would yield less than 1% of the nitrogen present in red maple leaf litter.

Introduction

The nitrate levels of soils in most Halifax County forests are extremely low. Nitrate levels of 2 kg per hectare were reported by the Nova Scotia Department of Agriculture for samples from the study site in the Waverley Game Sanctuary.Such low concentrations of this nutrient may limit productivity in this area. Therefore, it is important to reveal the sources of nitrogen that operate in these forest ecosystems.

Nitrogen fixation by living organisms is an important source of reduced nitrogen in ecosystems (Kelly & Becker 1975). The process involves the reduction of atmospheric nitrogen to ammonia which is catalyzed by nitrogenase. Only prokaryotic organisms are capable of fixing dinitrogen and they are found in a variety of associations in the forest.

A preliminary survey of forest sites in the Waverley Game Sanctuary indicated that the nitrogen fixing lungwort (*Lobaria pulmonaria* (L.) Hoffm. **) appeared only to occur on *Acer rubrum* stems. The objective of this study was to estimate the quantity of nitrogen fixed by lungwort, assess its possible significance as a nitrogen input to the forest ecosystem, and investigate the implications of lungwort's occurrence on *Acer rubrum*.

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**Hereafter referred to as lungwort in this paper.

Literature Review

Biology of the Lichen

Lungwort is a foliaceous, corticolous lichen of the *Stictaceae*. The thallus is comprised of a lichenized ascomycete mycobiont with a chlorophycean and a cyanophycean phycobiont. The chlorophycean component has been determined as *Myrmecia* (Ahmadjian 1969), which forms a continuous layer throughout the thallus and the cyanophycean is *Nostoc*. The blue-green alga is arranged in compressed clusters in internal cephalodia (Jordan 1970). Lungwort produces both soredia and isidia, usually along the thickened portion of thallus, which make it easily recognized. This species is the most widely distributed and most commonly collected *Lobaria* in North America (Jordan 1973).

Nitrogen Fixation by Lichens

Lichens which possess a blue-green alga as a thallus component have the ability to fix atmospheric nitrogen (Millbank & Kershaw 1969; Henriksson & Simu 1971; Hitch & Stewart 1973). About 50 species have demonstrated this capability. Studies by Millbank and Kershaw (1970) first indicated that lungwort was such a species, and that fixation was limited to the internal cephalodia, implicating *Nostoc* as the fixing phycobiont.

Dinitrogen heavy isotope investigations by Millbank and Kershaw (1970) on *Peltigera aphthosa* indicated that the labelled nitrogen fixed by *Nostoc* in the cephalodia had been transferred to and was overwhelmingly present in the mycobiont. Virtually all of the nitrogenous compounds produced by *Nostoc* are translocated to the mycobiont, which could be of vital significance to the maintenance of healthy growth of the thallus (Millbank & Kershaw 1970).

Nitrogen fixation is highly dependent on the metabolism of the lichen, thus environmental conditions that affect the metabolic rate are reflected in the rate of nitrogen fixation (Millbank & Kershaw 1973). Lichens are opportunists; metabolism is highly variable and depends largely on the thallus water content and temperature. Thus when conditions are suitable for assimilation and synthesis, the processes become active and when conditions deteriorate they revert to a state of quiescence (Millbank & Kershaw 1973).

The most important environmental factor affecting nitrogenase activity is the thallus moisture content (Hitch & Stewart 1973; Kershaw 1974). Henriksson and Simu (1971) reported the ability of *Peltigera* to recover its nitrogenase activity after prolonged periods of desiccation. The recovery rate is rapid after short drought periods, but recovery takes progressively longer as the drought period is extended (Kershaw & Dzikowski 1977). Kershaw (1974) found that each of the 12 species of lichen used in his study had different patterns of fixation response to changing levels of thallus moisture. These patterns were correlated with the ecological preferences of each species.

Another significant environmental factor concerning nitrogenase activity is temperature (Millbank & Kershaw 1969; Hitch 1971). Maikawa and Kershaw (1976) studied the temperature dependance of nitrogenase activity in *Peltigera canina*. They found that the optimum temperature varied seasonally with ambient temperatures. Subsequent work by Kershaw (1977) showed that lichens are able to photosynthetically acclimatize to lower temperatures. Considering the relationship between rates of metabolism and N_2 fixation, this may explain the seasonal changes in optimal fixation temperatures. Kelly and Becker (1975) indicated an optimum fixation temperature of 30°C for lungwort samples collected in North Carolina. No

activity was detected at 10°C. The seasonal variation in activity with temperature was not studied, and water-saturated samples were incubated at various temperatures without acclimatization periods.

The final major nitrogenase parameter is the level of light intensity (Kallio et al. 1972; Kallio 1973). Stewart (1965) showed that *Nostoc* was capable of some dark N_2 fixation, but it was considerably less than in light. MacFarlane et al. (1976) examined the interaction of light/dark periods in relation to the nitrogenase activity in *Peltigera polydactyla,* and calculated there was an immediate 50% decrease in activity when the lichen was darkened. There was a progressive further decrease with time. Results indicated a lag phase in activity upon re-illumination before it recovered to its original level.

The influence of light intensity on nitrogenase activity in lungwort was tested by Kelly and Becker (1975). Activity was shown to increase when the lichen was exposed to light intensities in excess of typical values found in shaded forests. Light saturation was never observed in the tests and is presumed to be above the maximum intensity used in the study (1200 Einsteins m^{-2} sec^{-1}). Kelly and Becker attributed the high saturation for lungwort to the embedded cephalodia.

Lichens as a Nitrogen Source

Nitrogen-fixing lichens have been shown to be important nitrogen contributors to their surroundings, especially in arctic and sub-arctic regions where they are the primary source of reduced nitrogen (Alexander & Schell 1973). Denison (1973) estimated that *Lobaria oregana* on tall Douglas fir contributes from 2.2 to 11.2 kg $ha^{-1}yr^{-1}$ to the forest. Becker et al. (1977) calculated a total nitrogen input of 1.23 g $ha^{-1}yr^{-1}$ for all nitrogen fixing corticolous lichens in a North Carolina oak-hickory forest.

According to Denison, nitrogen fixed by the lichen is released in several ways to the forest. The chief route of contribution is through decomposition processes such as bits of old thallus breaking off. Most of this removal is caused by the peeling action of rain, snow, and ice. The rapid nitrogenase rate probably enables more nitrogen to be fixed than the lichen can utilize for new growth (Denison 1973). This soluble excess of nitrogenous compounds may be leached with other metabolites from the thallus after rewetting owing to increased permeability and structural damage to cell membranes during desiccation (Farrar 1973; Crittenden & Kershaw 1978). Thus nitrogen may be washed down the tree by precipitation.

Stemflow

The proportion of rainfall intercepted by trees can be divided into 3 components: tree surface absorption, drips from leaves and branches (throughfall), and water flow down the trunk (stemflow). The relative proportion of the 3 components of interception depends on the quantity and duration of precipitation and the forest structure. The percentage of intercepted precipitation which reaches the ground surface as stemflow varies with tree species and is related to bark characteristics and the angle of branching. Rainwater becomes enriched in certain elements derived from trees (Kittredge 1948; Will 1955; Voigt 1960). It is well documented in the literature that losses of some elements from above-ground plant parts may occur by leaching (Tamm 1951). Gersper and Holowachuk (1971) produced evidence that the initial flush of stemflow has a higher concentration of elements than any subsequent flow during the same rainstorm. They attribute this to washing of particulate matter from the foliage and stems by the initial precipitation.

The quantitative contributions of stemflow to the nutrient cycle are small (Voigt

1960; Mahendrappa 1974). Voigt (1960) observed differences in stemflow distribution under different tree species and found the influence of stemflow was limited to a small distance (about 30 cm) from the tree stem. Zinke (1962) and Gersper and Holowachuk (1971) have also concluded that quantitative variations in the physio-chemical properties of soils near the trees have been caused by stemflow.

Mahendrappa (1974) studied the chemical composition of stemflow from some eastern Canadian tree species. His results indicated that stemflow of *Acer rubrum* had a higher pH (4.8) than stemflow of *Picea resinosa, Abies balsamea,* and *Pinus strobus.* His work is in agreement with Tamm (1951) and Voigt (1960) in indicating that rain seemed to leach more nitrogen from hardwoods than from softwoods. The estimated annual quantity of nitrogen returned to the ground by stemflow from *Acer rubrum* was 0.066 g $stem^{-1}$. The trees in this 55-year-old stand had an average height and DBH* of 16.8 m and 23 cm respectively.

Ecology of Corticolous Lichens

In forested regions, lichen communities form a complex mosaic that is broadly correlated with forest composition and tree density (Kershaw 1964). There are many factors implicated in determining the distribution of corticolous lichens within forests. The availability of water and a suitable substrate are the chief considerations (Barkman 1958). Hale (1955) concluded from a study of contiguous woodlots that about 60% of the variation in lichen communities could be caused by substrate factors and 40% by microclimate.

Lichens are termed poikilohydric as they passively follow the fluctuations of atmospheric humidity. Lichens do not have specific organs for the absorption or transpiration of water. Absorption of water into the hyphal walls occurs rapidly. Full saturation can be achieved in 1 to 4 minutes for foliose thalli immersed in water (Smith 1961). Water is easily lost by evaporation from the thallus surface. Field observations have shown that saturated thalli dry out within a few hours in dry weather (Blum 1973).

Foliose lichens possess the ability to absorb water vapor from the atmosphere (Pavillard 1939); however, it requires several days to equilibrate and only 50 to 75% of the potential levels of saturation can be obtained. The maximal saturated water content of lichens under natural conditions is achieved only during rain showers and for short periods thereafter (Blum 1973). Atmospheric humidity, however, is an important factor influencing water loss through evaporation (Barkman 1958). Relative humidity is known to vary with forest density, and with proximity to the forest floor.

The drought resistance of the genus *Lobaria* was examined by Lange (1953). The thalli endured 24 weeks in a desiccator without signs of visible damage.

The role of the bark supporting lichen thalli is complex. "It is open to question whether lichens derive any nutriment from their support at all" (Brodo 1973). It is clear that lichens have substrate preferences. The extent to which a lichen is restricted to a narrowly defined substrate type can be called its substrate specificity (Barkman 1958). The most important bark properties that govern lichen occurrence are texture, water relations, chemistry, and stability.

The texture of bark affects the ease of colonization. Lichen diaspores can become trapped and begin to develop on rough surfaces more easily than on smooth surfaces (Brodo 1973).

Differences in lichen floras have been attributed to bark-water capacities (Hale 1955; Culberson 1955; Margot 1965). Harris (1971) found that substrate moisture acts

*diameter at breast height (1.3 m)

in the hydration of the thalli, thereby influencing the lichens' rate of photosynthesis and respiration. Lungwort has been reported to favor *Quercus* in coastal regions but *Fagus* in inland areas because of the high water capacity of *Fagus* bark (Barkman 1958). This supports the theory that lichens respond to bark characters, not to tree species. In analyzing epiphytic vegetation on *Acer* and *Pinus,* des Abbayes (cited by Brodo 1973) concluded that *Acer* did not only retain more moisture than did *Pinus,* but liberated it more regularly and made it available to lichens over a longer period of time and at higher levels.

Of all aspects of substrate chemistry which might be considered, pH has been the most studied and discussed. The acidity or alkalinity of the substrate can act indirectly on lichen thalli in numerous ways. The "availability" of various minerals and organic substances varies under different pH regimes. Diffusion rates may change at different pH's, and some substances are more toxic under acidic conditions (Brodo 1973). A direct influence of pH might operate through the effects on the activity of enzyme systems in the lichen (Brodo 1973). Whatever the mechanism of influence, studies by Culberson (1955) and Kershaw (1964) have demonstrated that the distribution of some lichens and lichen communities is strongly correlated with substrate acidity. The presence of lichens on a tree trunk modifies the pH (Kershaw 1964). Uncolonized areas have lower pH's than those that are colonized. Conifers generally have a lower pH than hardwoods. In particular, the buffering capacity of bark is important.

The final substrate parameter considered is the stability of the bark. Since lichens are slow growing, they are generally more abundant on trees whose bark is seldom sloughed (Becker et al. 1977).

Most of the bark substrate qualities (texture, water relations, chemistry, and stability) vary with the vertical distribution on the tree stem. The bark texture generally becomes coarser at the base of the trunk. Studies by Kershaw and Harris (1971) indicated that the vertical distribution of *Parmelia* on tree trunks was determined by water availability. The pH also varies at different levels on the tree stem, the upper layers having a higher pH than the lower (Kershaw 1964).

Competitive interactions occur among epiphytic lichens. Competition may be categorized as suffocation, competition for light, and chemical action (Barkman 1958). The chief competitors at the lower trunk levels are bryophytes. Bryophytes growing in dense mats sometimes overgrow lichens (Hale 1967). Epiphytes belonging to higher strata tend to crowd out those of lower strata by over-shadowing them (Barkman 1958).

Generally, as the tree and branches grow and the crown and canopy of the forest develop, the lower parts of the tree are shaded and lichens become distributed along the trunk in response to their environmental needs. As a result, a characteristic vertical pattern is produced (Kershaw 1964).

Description of the Study Area

All field work for this study was conducted in the Waverley Game Sanctuary along a 3-km section of the Old Guysborough Road. The Sanctuary is situated 20 km northeast of Halifax and comprises 52.3 km^2 of crown and privately owned land.

The underlying rock in the study area is quartzite of the Gold-bearing Series. The thin layer of soil covering the rocks is categorized as steepland till. The soil has a sandy-clay-loam texture with coarse, stony fragments. Soil analysis by the Nova Scotia Department of Agriculture indicated that the soil is moist, acidic (pH 3.4), and about 45% organic matter.

The area is covered by a second-growth forest of moderate density (Lewis 1963). In the study area, the most frequent tree species were *Picea rubens, Abies balsamea,* and *Acer rubrum,* with occasional occurrences of *Pinus strobus, Betula papyrifera,* and *Picea mariana.*

Materials and Methods

Ecology of Lungwort

Ten 400-m^2 quadrats were randomly chosen at least 30 m from the Old Guysborough Road.

Preliminary observations indicated that lungwort occurred predominantly on *Acer rubrum* in this locality. To substantiate this observation, stems on each plot were systematically examined and a census of all *Acer rubrum* stems with a DBH greater than 5 cm was conducted. If the lichen occurred on a stem, the tree species was noted and its DBH measured to give an approximate estimate of age. The vertical distribution was also recorded. To make a quantitative estimate of lungwort coverage, at least 8 randomly chosen trees on each plot (where available), were tested for percent cover using the cover pin technique (Goldsmith & Harrison 1976). Since lungwort thalli tend to overlap, and the cover pin technique only quantified in 2 dimensions, a correction factor was calculated from the frequency of the cover pin hitting areas of thallus overlap. Because of height limitations, the percent cover determined by this method applied only to the lower 2 m of the stem. Above this height, the coverage was estimated by visual inspection.

To determine whether the alteration in microclimate resulting from *Acer rubrum* stump sprouting affected lungwort coverage (per stem), the number of stump sprouts was recorded (Prager & Goldsmith 1977).

Nitrogenase Activity of Lungwort

The nitrogenase activity of the lungwort was measured in the field using the acetylene reduction technique (Hardy et al. 1973). Nitrogenase catalyzes the conversion of acetylene to ethylene and thus the quantity of ethylene provides a direct measure of nitrogenase activity. A 3.2:1 molar ratio of ethylene produced to nitrogen fixed was used to estimate N_2 fixation (Hardy et al. 1973).

Portions of intact lungwort mats were removed from stems and placed in 1000-ml Mason jars with a serum cap embedded in the lid. The appropriate amount of acetylene was injected to produce a 10% atmosphere of this gas. The incubation jars were then placed on the forest floor at the collection site.

Gas samples were taken in evacuated blood-sampling tubes. An initial ethylene reading was taken 5 min after acetylene injection. Data from time course experiments indicated that a l-h incubation period was sufficient to estimate ethylene production. After the gas samples were collected, the lungwort was transported to the laboratory where the thallus surface area, fresh weight, and oven dry weight (24 h at 90°C) were determined.

Gas samples were analyzed by gas chromatography on a Carle Model 9500 flame ionization gas chromatograph, equipped with a 0.32 x 50 cm, 80/100 mesh Poropak T column. Nitrogenase activity was measured in the months of April, May, June, September, and October of 1978 and February of 1979.

Stemflow Collection

To measure the amount of nitrogen that may be added to stemflow as it passes

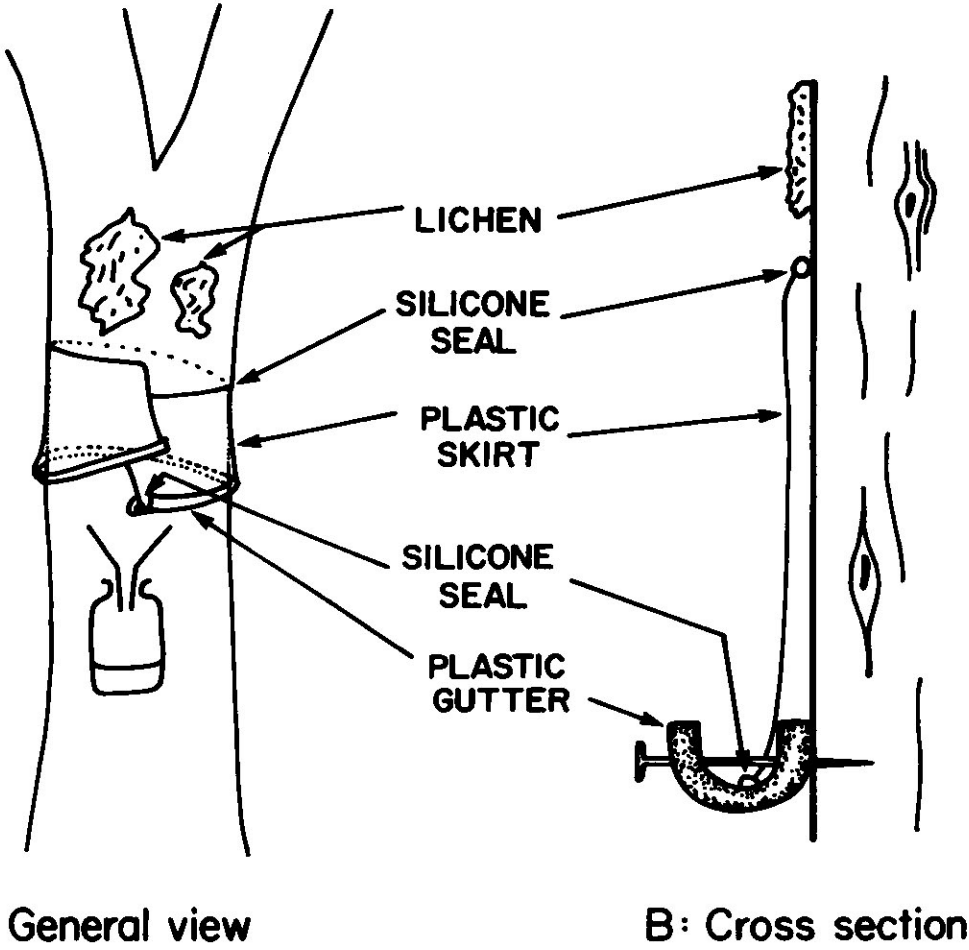

Fig. 1. Apparatus for collection of stemflow from *Acer rubrum* trunks. Another plastic skirt was fastened over the area of lungwort colonization to collect samples that had not run over the lichen.

over the trunk, and determine the possible contribution by lungwort, throughfall and stemflow samples were collected and analysed for nitrogen content.

The apparatus used to collect stemflow consisted of a gutter formed by a split 1.5-in d plastic tubing fastened around the circumference of the trunk. A funnel with a 1/16 in and a 100 μm filter screen was attached to the lower end of the gutter. The funnel was 7-cm d so very little throughfall was collected (10%). An acid-washed 250-ml plastic collection bottle was taped securely to the funnel. Above this assembly, a polyethylene skirt was adhered to the stem and gutter with silicone (Fig 1). Another longer skirt (about 0.6 m), could be placed over the lungwort to prevent stemflow from passing over lichen thalli. Two such devices were constructed on *Acer rubrum* stems where lungwort only occurred as high percentage cover band on the trunk.

Throughfall was collected in troughs made from 3-m sections of split, 5-in d PVC pipe. The trough channeled throughfall to the same type of filters and collection container used for stemflow.

Stemflow samples that had passed over lungwort and those that were prevented from doing so, were collected on the same tree from the same rainstorm. Throughfall samples were also collected at this time. Collection was made during each rainstorm. Samples were placed immediately in a cooler and frozen within 3 h of collection. The samples were stored at -3°C until they were analysed for nitrogen.

The Kjeldahl technique (Bremner 1965) was used to determine the total reduced nitrogen concentration in duplicate 50-ml aliquots from each collection.

For calculations, the volume of stemflow for *Acer rubrum* was taken to be 5.6% of total rainfall (Mahendrappa 1974). The average canopy cover for this species in the study area was estimated at 7.4 m^2. Annual nitrogen requirements for *Acer rubrum* (Table I) were calculated from data of Young and Carpenter (1967).

Table I. Mean annual nitrogen requirements for a 30-35′ *Acer rubrum*

Foliage	2.0 gN
Branches	1.3 gN
Stems	4.6 gN
Roots	1.9 gN
Total	9.8 gN

Estimation of Annual Quantity of Nitrogen Fixed

Estimation of annual nitrogen fixation by lungwort was calculated by combining environmental parameters governing nitrogenase activity, determined from both this study and from the literature, with meteorological data compiled by Environment Canada (1978). The calculation considers lichen-water relations (wetting and drying rates) and monthly variations in daylight hours and temperature.

To estimate the moisture content of the thallus for a given rainfall, data produced by Harris (1972) were used. His results indicate that an average of 5.3 mm of rain is necessary to saturate the bottom 2 m of corticolous lichen situated on a deciduous tree during the winter when no canopy is present; 8.3 mm of rain is necessary when the canopy is present. These values were used in the calculation, and after each rainfall in the calendar year 1978, lungwort thalli were assumed to be saturated. The period of canopy cover was designated from May to October.

The drying rates for lungwort were obtained from a linear regression calculated from values of lichen percent moisture after various periods of desiccation following saturating rain events (Fig 2).

Seasonal variation may be expected in evaporation rates. In winter months low temperatures would decrease rates. However, the loss of full canopy increases wind velocities within the forest thereby increasing evaporation rates. In these calculations, the same evaporation rates were used for all seasons. The nitrogenase activity rate for each day following saturation was determined from the ethylene-production-moisture graph (Fig 3).

It was assumed that N_2 production is restricted to daylight hours. To account for the seasonal variations in day length at 45°N latitude, median values for each month as provided by Environment Canada (1978) were used.

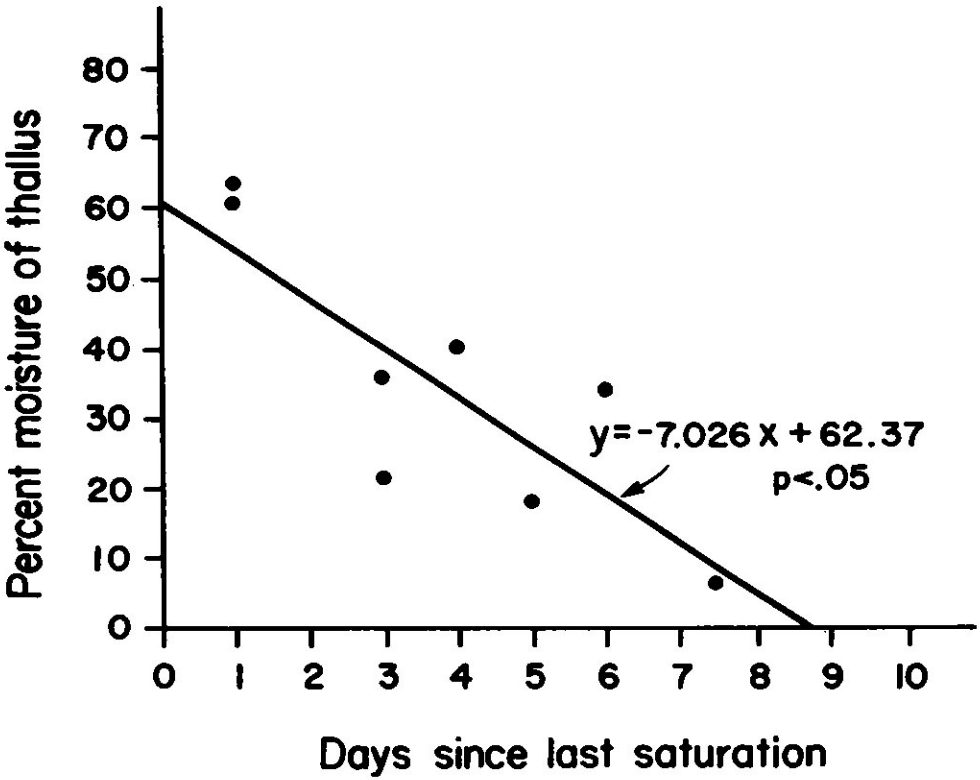

Fig. 2. Moisture content of lungwort thalli collected from *Acer rubrum* at various intervals after saturating rainfall, June, 1978.

Table II. Summary of *Acer rubrum* census

Quadrat (400 m^2)	No. *Acer rubrum* stems (over 5 cm DBH)	No. *Acer rubrum* stems with lungwort
1	21	9
2	3	2
3	17	9
4	36	4
5	16	0
6	52	10
7	10	2
8	12	3
9	36	7
10	25	6
Total	228	52.0

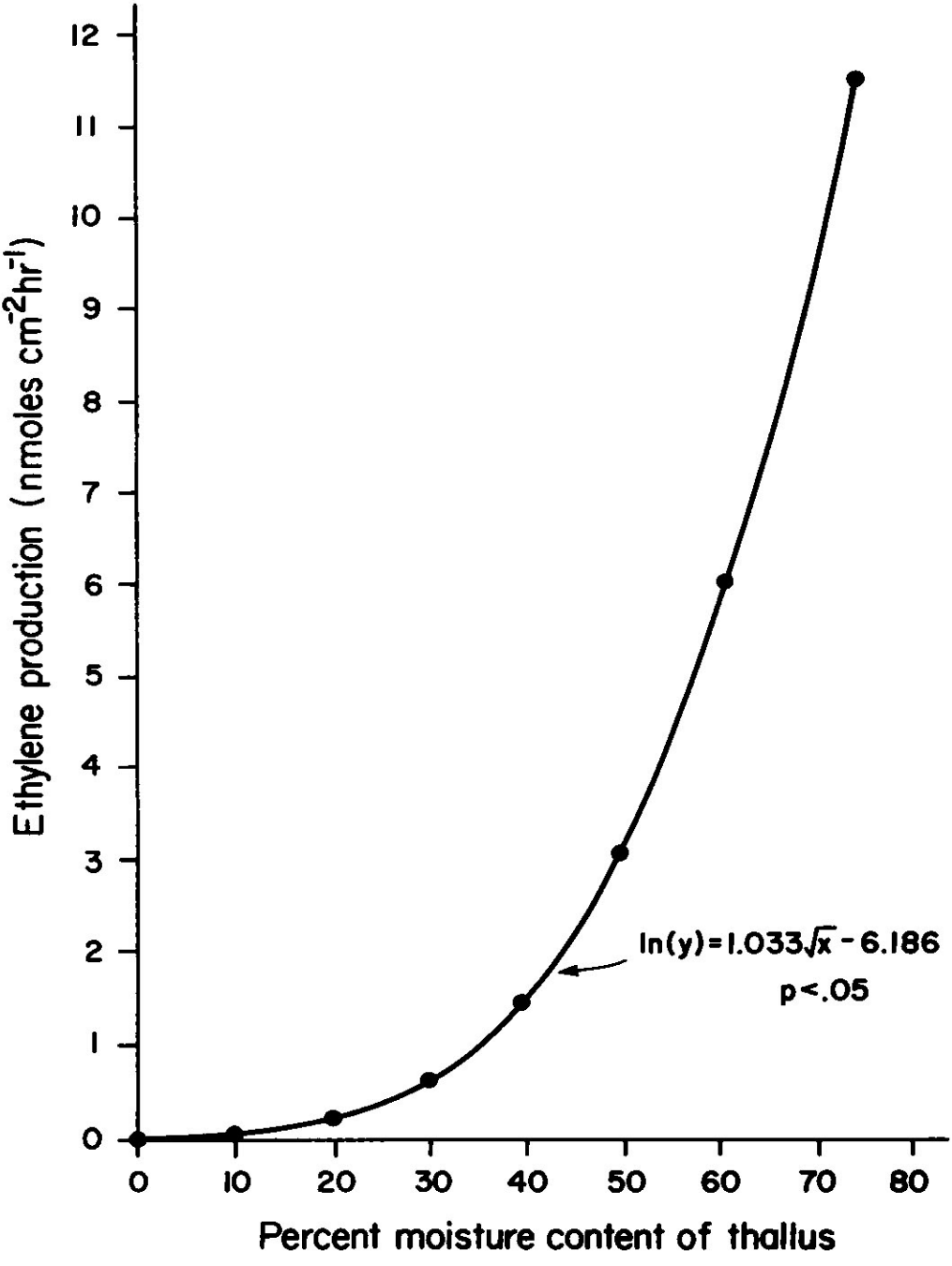

Fig. 3. The relationship between nitrogenase activity and thallus moisture content estimated from data of June, 1978.

The sum of the ethylene production values for each month was multiplied by this value to give the total amount of fixation for that period. It was assumed that fixation which occurred in darkness was equal to the amount lost during the nitrogenase lag phase experienced upon re-illumination.

The effect of seasonal temperature variations on nitrogenase activity was approximated in the calculation by multiplying the total N_2 fixation for each month by a temperature correction factor. This factor was determined from a Q10 value calculated from the differences in nitrogenase activity at 3° and 15°C (Fig 4) when moisture contents were equivalent, and utilized the mean daytime temperature for each month. The temperature corrected, total ethylene production figures for each month were converted to nitrogen fixation values and summed (Table III). The Q10 value was used without reference to a critical minimum temperature below which no activity occurs.

Results

Ecology of Lungwort

Thorough inspection of all 10 quadrats indicated that lungwort occurred only on *Acer rubrum* stems (Fig 5-8 and Table II). The density of *Acer rubrum* was found to vary considerably between quadrats. Values ranged from 3 to 52 stems pvr 400 m^2. Lungwort occurred on 22.8% of *Acer rubrum* in the study area. Coverage values for individual stems also varied considerably. The mean value for coverage of stems with lungwort is 0.207 m^2 per stem. Stems with a larger DBH had a higher mean frequency of lungwort occurrence (Fig 5), but the 20 to 25 cm DBH class had the greatest mean coverage value (Fig 6). Lungwort coverage per stem was greatest in cases where 2 or 3 stems were produced from a stump (Fig 7).

The vertical distribution of lungwort indicates that it occurs close to the forest floor. Sixty percent of lichen coverage was within 1 m of the ground and a 10-m height encompassed 96% of coverage (Fig 8).

Table III. Monthly estimates of nitrogen fixed by lungwort (1978)

Month	μg N fixed cm^{-2} lichen	gN fixed ha^{-1} forest
January	1.01	0.128
February	0*	0
March	1.40	0.178
April	3.58	0.455
May	4.08	0.519
June	5.46	0.693
July	3.97	0.505
August	0*	0
September	3.75	0.477
October	2.66	0.338
November	.76	0.096
December	1.12	0.142
Year Total	27.80	3.531

*The actual values could be as high as 1.01 for February and 3.75 for August.

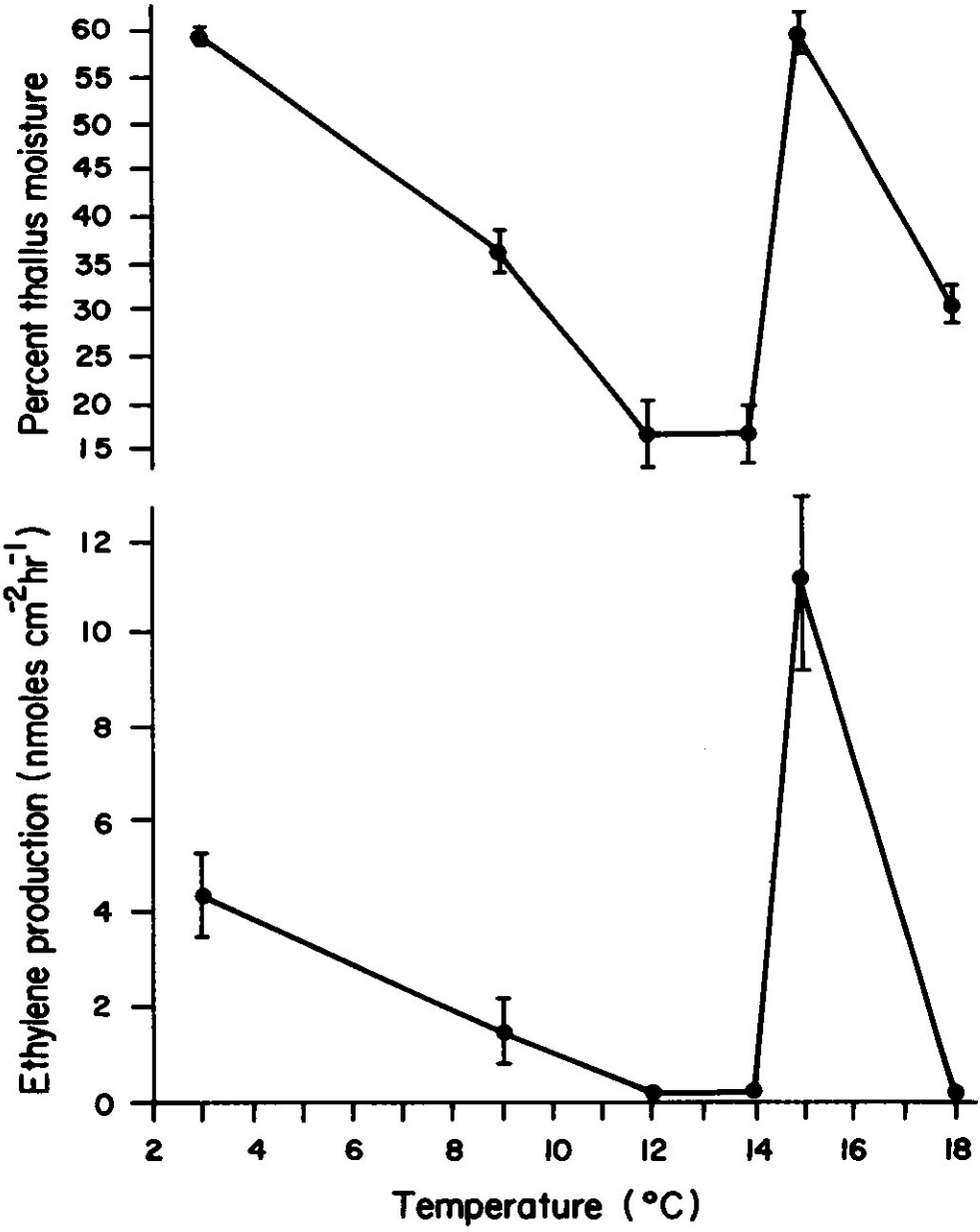

Fig. 4. Thallus moisture content (top) and nitrogenase activity (bottom) on days of different temperature. A Q_{10} factor for nitrogenase activity was calculatged from the nitrogenase activities at 3° and 15°C, when moisture contents were equivalent. Bars indicate standard deviations.

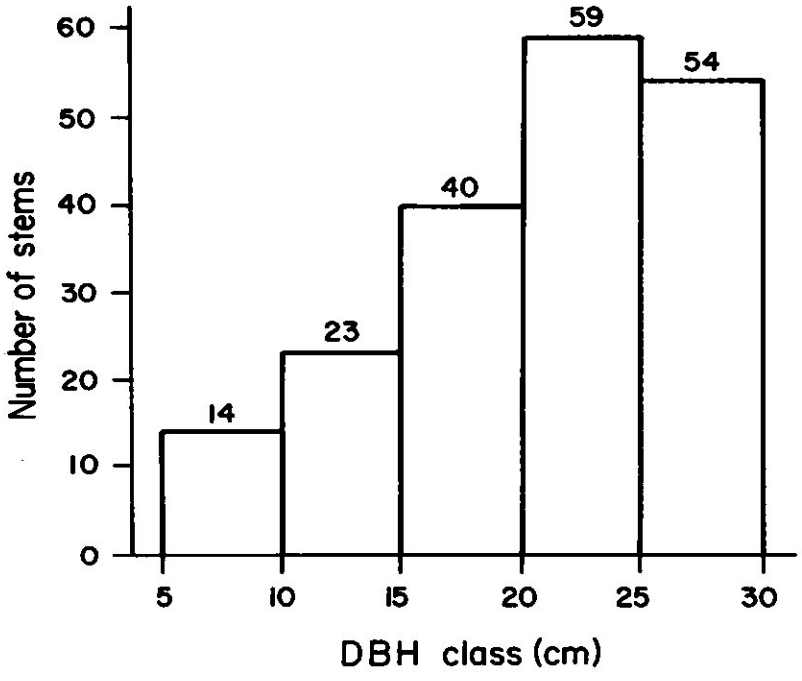

Fig. 5. Frequency of lungwort on *Acer rubrum* stems of different diameters.

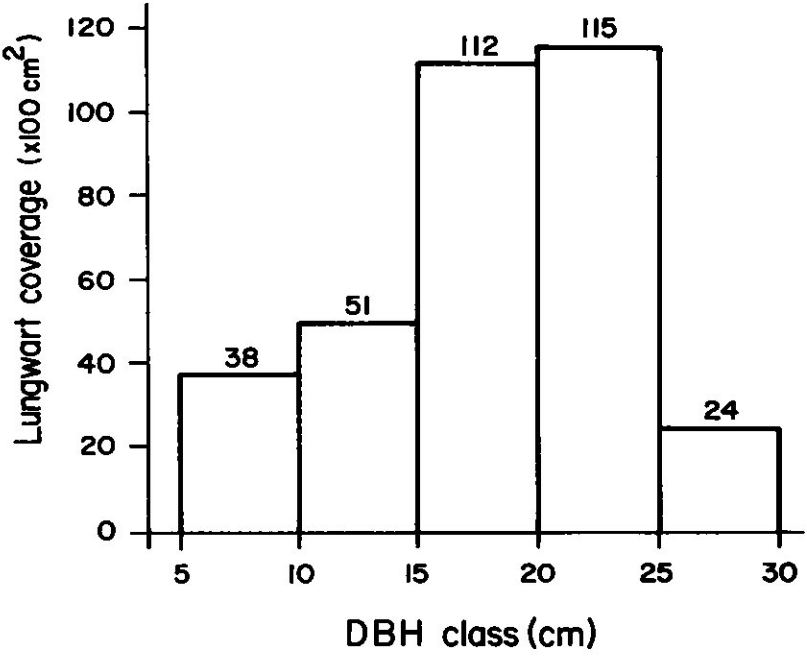

Fig. 6. Lungwort coverage on *Acer rubrum* stems of different diameters.

From the data collected in the study area, it is estimated that lungwort coverage in this area is 5.075 m^2 or 12.7 m^2 per hectare.

Nitrogenase Activity of Lungwort

The results of the acetylene reduction assays were extremely variable. Figure 4 indicates that the activity is closely correlated with the moisture content of the thallus. Figure 3 illustrates the calculated relationship between thallus moisture and nitrogenase activity for lungwort collected in June. The enzyme was found to be active in spring, autumn, and winter with the second highest activity reading occurring in February 1979 after a period of intense rainfall.

Stemflow

Results of the analysis for nitrogen content of stemflow and throughfall samples are presented in Table IV. These data support the contention that nitrogen is added to the rainfall as it flows down the trunk.

Table IV. Kjeldahl nitrogen in stemflow samples collected from *Acer rubrum* stems with lungwort cover, and throughfall samples collected under the *Acer rubrum* canopy

Collection Date	Quadrat	Throughfall mgN/50 ml	Stemflow no Lichen mgN/50 ml	Stemflow over Lichen mgN/50 ml	Apparent nitrogen accumulation mgN/50 ml
08/06/78	3(A)	107.7	103.9	138.8	+34,9
08/06/78	4(B)	43.3	57.2	76.2	+19.0
13/06/78	9(C)	27.5	75.5	178.2	+131.1
13/06/78	10(D)	22.0	75.5	73.3	-2.2*

*This negative value possibly an experimental error.

Discussion

Nitrogenase activity was observed to vary considerably between sample assays. However, a close correlation between moisture content and activity was observed. Results are in agreement with work done on other lichen species by Hitch (1971), Hitch and Stewart (1973), and Kershaw (1974) in that thallus-moisture content is the most important environmental factor affecting activity. Recovery of activity after periods of desiccation were also observed.

Temperature was also found to influence nitrogenase activity. Comparison of activity from June and February with comparable thallus-moisture contents showed a 68% drop in nitrogenase activity with a 12°C drop in temperature (Fig 4). Winter results for lungwort activity contrast with those of Kelly and Becker (1975) who detected no nitrogenase activity in temperatures under 10°C.

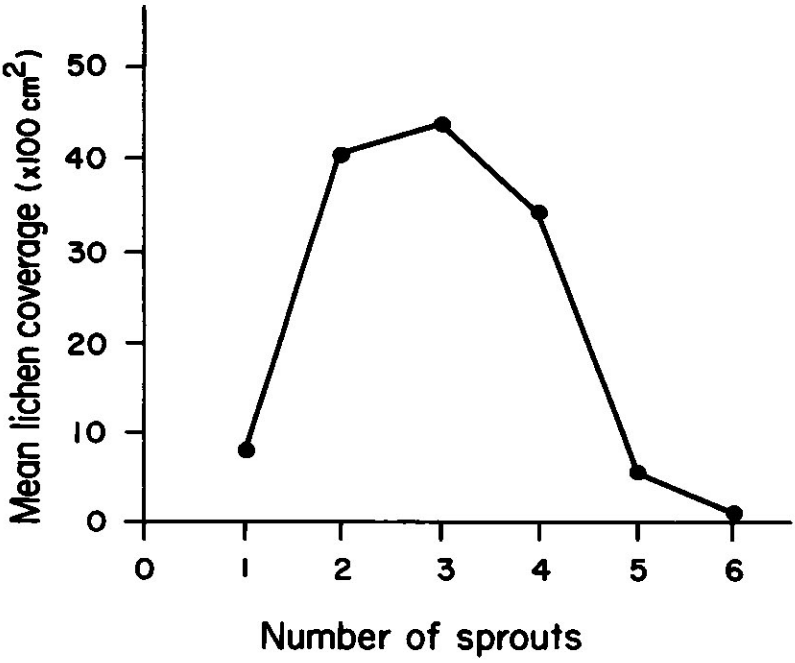

Fig. 7. Lungwort coverage in relation to number of *Acer rubrum* stump sprouts.

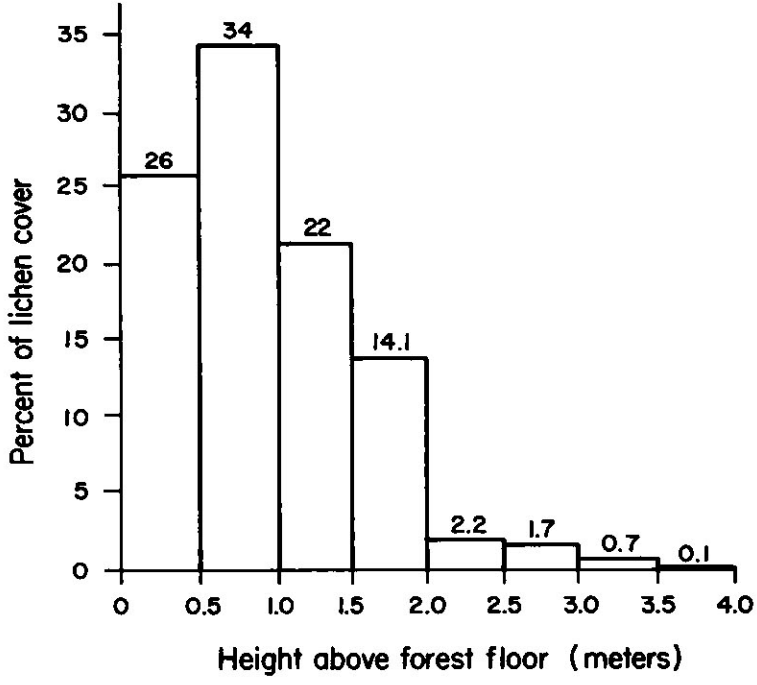

Fig. 8. Percentage of total lichen cover in successive 0.5-m intervals above the forest floor.

Monthly climatic variations cause N_2 fixation results to be sporadic. Estimated N_2 fixation for February is indicated as zero in Table III because the month was cold with most of the precipitation occurring as snow. However, the readings which were obtained in that month suggest that at least on some occasions high rates of N_2 fixation are possible. Therefore, the yearly total is calculated assuming the lowest possible value figure although likely it is as high as that for January. Estimated N_2 fixation for August was zero as rainfall was insufficient to saturate the thallus. The period of greatest fixation predicted by the calculations is in the late spring and early summer.

Annual predictions of nitrogenase activity for lungwort indicate that about 3.53 gN ha^{-1} could be fixed annually. Soil analysis for this region indicate a nitrate value of 2 kg ha^{-1} (N.S. Dept. Agric.). Atmospheric washout adds from 2 to 10 kg of nitrogen per hectare (Russel 1961). Thus 3.53 gN ha^{-1} represents 0.176% of the nitrate-nitrogen in the soil, and the minimum amount of nitrogen added by precipitation is similar. 3.53 gN ha^{-1} yr^{-1}, though low, is 2.9 times greater than the fixation reported by Becker et al. (1977) for all nitrogen-fixing lichens in a North Carolina oak-hickory forest.

Since the influence of stemflow on soil properties is limited to 30 cm from the tree stem (Voigt 1960), we investigated whether the N_2 fixation by the amount of lungwort present on 1 tree, is of significance to the nitrogen budget of that tree. For these calculations, an average and the maximum lungwort coverage values for the stems in the study area were used. The average coverage of lichens could only fix N_2, equivalent to about 0.6% of the annual increase in nitrogen content of the stems. The maximum amount of lungwort on a stem could possibly fix N_2 equivalent to 4.9% of the nitrogen content of the foliage. Thus fixation by lungwort appears to be of limited significance to the nitrogen budget of the *Acer rubrum* tree. Results of the stemflow and throughfall analysis for nitrogen are in agreement with Kittredge (1948), Will (1955), and Voigt (1960) in that rainwater samples which had flowed over the stem became enriched in nitrogen. The nitrogen concentration of stemflow was found to vary considerably between *Acer rubrum* stems (Blacklock 1979).

The collection at site D was different from the others in that stemflow which did not pass over lungwort was collected first (Table IV). This was the only site where there was no net increase in nitrogen when stemflow was allowed to pass over lungwort. Gersper and Holowachuk (1971) found the initial flush of stemflow has a higher concentration of nutrients than subsequent flow resulting from washing of particulate matter from the foliage and bark. Hence it is possible that the estimated nitrogen contribution by lungwort to stemflow on the tested stems is biased with estimates of accumulation for quadrats A to C being too high.

If it is assumed that the mean difference in nitrogen in stemflow samples results from leaching from lungwort and the leaching rate is constant, then 0.114 g yr^{-1} of nitrogen may be contributed to stemflow by the average amount of lungwort on an *Acer rubrum* stem. The total estimated input of nitrogen by stemflow is .218 gN per tree which is about 2.2% of the tree's annual nitrogen addition to biomass. Thus stemflow does not appear to be a significant source of nitrogen for *Acer rubrum* in this area.

According to Denison (1973), the major flux of nitrogen from lichens to the ecosystem is via litter fall. Using a 2.7% nitrogen content (dry wt) for lungwort and a conversion of 1 g lungwort being equivalent to 40 cm^2, the total amount of nitrogen incorporated in lungwort biomass is 86 g ha^{-1}. Using even a generous decomposition rate of 10% (Barkman 1958), it is evident that lungwort litter fall would not be an important source of nitrogen in the forest, as it would amount to only 0.75% of the nitrogen available from *Acer rubrum* leaf litter.

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