SHORT-TERM EFFECTS OF A SIMULATED ACID RAIN UPON THE GROWTH AND NUTRIENT RELATIONS OF *PINUS STROBUS*, L.[1]

TIM WOOD, Research Assistant, Yale University, School of Forestry and Environmental studies, New Haven, Conn. 06511.
F. H. BORMANN, Professor of Forest Ecology, Yale University, School of Forestry and Environmental studies, New Haven, Conn. 06511.

ABSTRACT

Acidified precipitation may affect the productivity of forests by altering the availability of plant nutrients of by affecting the ability of trees to absorb and assimilate those nutrients. In this study, the short-term effects of simulated acid rain (pH range 5.6 - 2.3) upon the growth and nutrient relations of Eastern White Pine seedlings (*Pinus strobus*, L.) grown in a sandy loam soil were examined. Soil chemistry, soil leachate chemistry, seedling tissue chemistry, and seedling productivity were monitored.

In spite of increased leaching of cations from the soil, resulting in near depletion of available K^{+1}, Mg^{+2}, and Ca^{+2} at pH 2.3, seedling productivity increased with acidity through the 20-week experimental period. Foliar nitrogen concentrations indicate that fertilization with NO_3^{-1}, added to the rain as HNO_3, may have caused the growth increase. Implications of cation losses and NO_3^{-1} fertilization are discussed.

The potential effects of acid precipitation on forest ecosystems are numerous and wide-ranging. Recent investigations suggest that mounting inputs of hydrogen ion via rainwater may cause: 1) A decrease in the pH and base saturation of forest soils (5) resulting from increased leaching of metallic cations from exchange surfaces (6); 2) An increase in foliar leaching from the forest canopy (4, 10); 3) Abnormal morphogenesis of, and injury to, plant tissue, especially leaves (2, 9); and 4) A reduction in forest productivity (1, 3, 7). The consequences of mounting inputs of SO_4^{-2}, NO_3^{-1}, and Cl^{-1}, the primary anions associated with acid rain, have received little attention.

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In the past, these effects have been studied, for the most part, individually. As a result, compounded stresses and relationships between stresses may have gone unnoticed and unmeasured. In this paper, we describe an integrated study of some of the effects of a simulated acid rain upon the growth and nutrient relations of Eastern White Pine seedlings (*Pinus strobus*, L.) grown in a sandy loam soil. Soil chemistry, soil leachate chemistry, seedling tissue chemistry, and seedling productivity are examined.

METHODS

On October 18, 1973, each of 125 three-inch plastic pots containing 180 ± 6 grams of sandy loam soil* were planted with four white pine seeds. The seed was collected from a single tree located in northwestern Connecticut. The pots, placed on a greenhouse bench under 3,000 Lux of supplimentary incandescent lighting (16 hr/day), were regularly irrigated with distilled water. No fertilizers were applied. Two weeks after seedling emergence, each pot was thinned to one seedling, and the population was randomly divided into five subsets of 25 plants.

Beginning on December 3, 1973 and continuing for the next 20 weeks, each subset was subjected to one weekly six-hour artificial acid rain adjusted to one of the following pH's: 5.6 ± 0.6, 4.0 ± 0.1, 3.3 ± 0.1, 3.0 ± 0.0, and 2.3 ± 0.0. To simulate the anion composition of rainfall in central New Hampshire, "rains" were solutions of sulfuric, nitric, and hydrochloric acids in the proportion by chemical equivalents, SO_4^{-2} (66%), NO_3^{-1} (24%), Cl^{-1} (10%). Analytical grade acids and glass distilled water were used. Rain drops (0.5 - 0.8 mm in diameter) were produced with a stainless steel rain nozzle. The seedlings, placed on a turntable, rotated slowly through the "rain" with each pot receiving approximately 190 ± 5 mls of solution per treatment (190 mls/week = 4.2 cm/week in comparison to an average of 2.5 cm of rain falling weekly in the northeastern U.S.). During treatment, plants were illuminated with 4300 Lux of fluorescent light.

Soil Leachate Chemistry: Four pots from each subset of 25 were randomly selected for soil leachate analysis. During every other treatment (beginning on the first week) leachate flowing from the bottom of these pots was collected, weighed to the nearest gram, and analyzed to determine K^{+1}, Mg^{+2}, and Ca^{+2} concentrations (atomic absorption

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*Physical Characteristics: Sand: 86%, Silt: 10%, Clay: 4%. Loss on ignition: 2.6%. Cation Exchange Capacity: 0.50 meq/10g soil.

analysis) and pH (electrometric analysis). A pot containing neither soil nor seedling, a control, was monitored identically. Net losses per treatment of K^{+1}, Mg^{+2}, and Ca^{+2} (mg) were calculated for each monitored pot by subtracting control cation losses from gross leachate losses.

At the conclusion of the experiment, the ten bi-weekly leaching losses were summed for each pot and doubled to estimate the total amount of each cation leached from each pot during the 20 treatments. Means and standard deviations for estimated total losses of the three metallic cations were calculated for each pH level and t-tests were computed between all treatment level pairs to detect significant differences in leaching. Differences were considered significant when $p \leq 0.005$.

Seedling productivity: Following the 20 treatments, the pine seedlings were examined for tissue damage and then harvested. Their roots were washed. The lengths of all fascicled needles of five randomly-selected seedlings from each subset were measured. Total plant weight and total needle weight were determined for each seedling following oven drying at 90°C. Means for each subset were subjected to t-tests to detect significant differences ($p \leq 0.005$) in productivity between pH levels (Fig. 1).

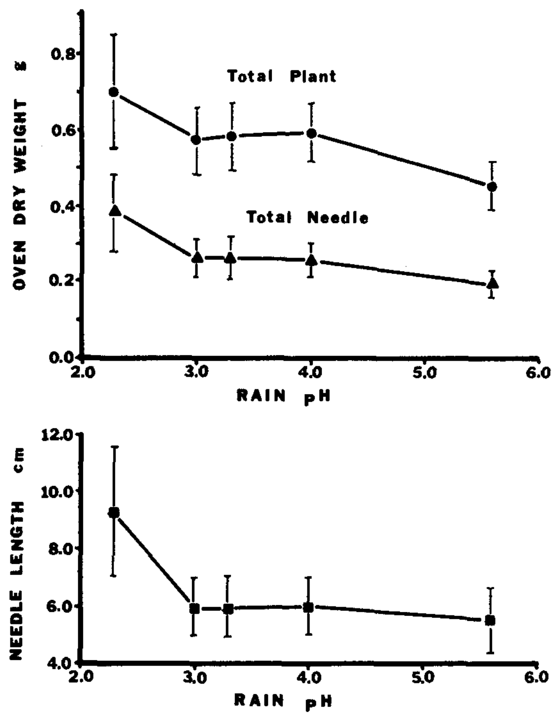

Figure 1. Seedling productivity as affected by rain chemistry. Mean oven dry total plant weight, mean oven dry total needle weight per plant, and mean fascicled needle length are shown.

Tissue Chemistry: Needles from the seedlings of each treatment level were pooled and two subsamples from each were analyzed for K^{+1}, Mg^{+2}, and Ca^{+2} using an H_2O_2 - H_2SO_4 digestion and atomic absorption analysis. Kjeldahl nitrogen concentrations were determined with the Kjeldahl digestion technique. Stem and root tissue were similarly pooled, subsampled, and analyzed. Nutrient concentrations and corresponding tissue weights were used to calculate mean weights of K^{+1}, Mg^{+2}, Ca^{+2}, and Kjaldahl N, assimilated per plant in each treatment level.

Soil Chemistry: The soil from each treatment was pooled and analyzed (two replicates) to determine pH (electrometric analysis), exchangeable K^{+1}, Mg^{+2}, and Ca^{+2}, (ammonium acetate extraction), Kjeldahl nitrogen (Kjeldahl digestion), and nitrate-nitrogen (phenoldisulfonic development (8)). A sample of untreated soil was also analyzed.

"Rain", leachate, soil, and tissue chemistry data were compiled into partial nutrient budgets for the five treatment levels (Figs. 3a - 3f). Estimates of soil weathering inputs of K^{+1}, Mg^{+2}, and Ca^{+2} were calculated using the following equation.

Weathering Input = Loss from Soil - Input to Soil - Declines in Exch. Cations = (Soil Leachate + Tissue Assimilation) - (0.0 input of cations) - (Initial - Final Exchangeable Cations)

RESULTS AND DISCUSSION

Growth analyses indicate that short-term productivity of the White Pine seedlings significantly increased as concentrations of H_2SO_4, HNO_3, and HCl were increased in the artificial rain. Total plant weight and total needle weight were significantly lower at pH 5.6 than at all higher acidities, and all growth measures were significantly higher at pH 2.3 than at all other levels (Fig. 1). No significant growth differences were observed in the pH range 4.0 to 3.0. The relationship between assimilated Kjeldahl nitrogen (Fig. 3f) and productivity (Fig. 1) through the five pH levels suggests that fertilization with HNO_3-nitrogen may have been an important growth determinant.

Tissue damage, necrotic needle segments up to 2 mm long, appeared on all seedlings exposed to pH 2.3 "rains". No other seedlings displayed visible injury. Decreases in growth over the 20-week period did not coincide with leaf damage, but continued destruction of photosynthetic tissue may, in the long run, affect reductions in productivity.

Gordon(2) showed needle dwarfism (needles growing to only one half their normal length) in pines resulting when developing foliage was misted with H_2SO_4 solutions adjusted to pH's below 4.0. Our work produced quite different results (Fig. 1). It is unclear why stunting did not result from our acid treatments. Increased needle growth was probably caused by NO_3^{-1} fertilization.

Figure 2 conceptualizes nutrient flow through and within our plant-

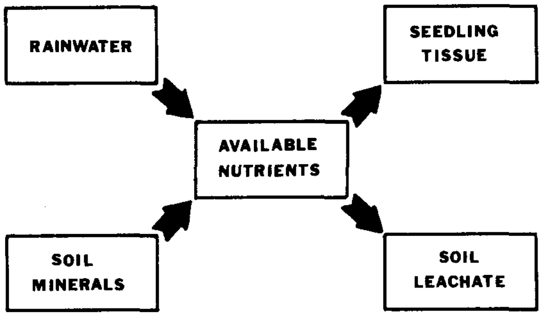

Figure 2. A simple model of nutrient flow through and within the plant-soil systems. Five nutrient reservoirs and the major fluxes between them are shown.

soil systems. Cation flux centers about the pool of exchangeable cations held at polar binding sites on soil particles and free within the soil solution. Additions may be made to this pool via "rainwater" input and soil mineral weathering. The pool may be decreased through plant uptake and leaching outputs. Figures 3a - 3f indicate that patterns in K^{+1}, Mg^{+2}, Ca^{+2}, and N flow through our systems were altered in several ways as the acid concentration of the "rain" was increased.

Inputs of H^{+1}, SO_4^{-2}, NO_3^{-1}, and Cl^{-1} were of course increased with rain acidity. Figure 3a shows the total input of nitrogen per pot for the 20 treatments increasing logrithmically through the pH range. No K^{+1}, Mg^{+2}, or Ca^{+2} were added with "rain".

Figure 3. Effects of rain chemistry upon the nutrient pools and nutrient flows through and within the plant-soil systems.

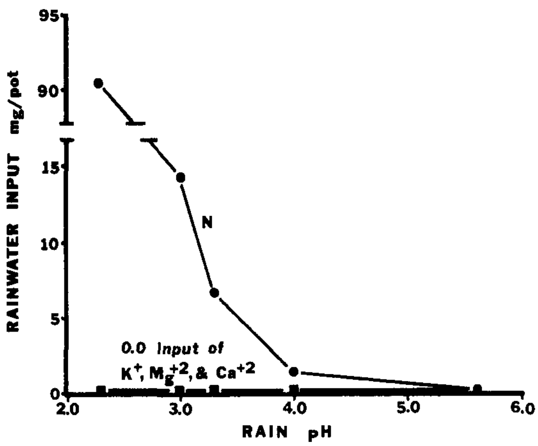

Figure 3a. Total inputs of N, K^{+1}, Mg^{+2}, and Ca^{+2} with rainwater after 20 treatments.

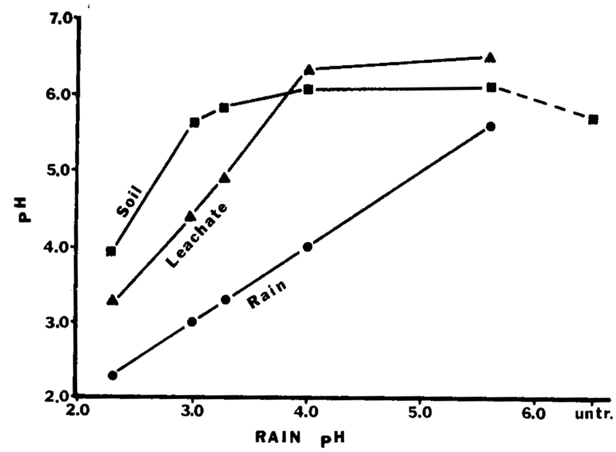

Figure 3b. Mean pH of rain and soil leachate through the 20 week experimental period, and soil pH before and after the 20 weeks (Untr = untreated soil).

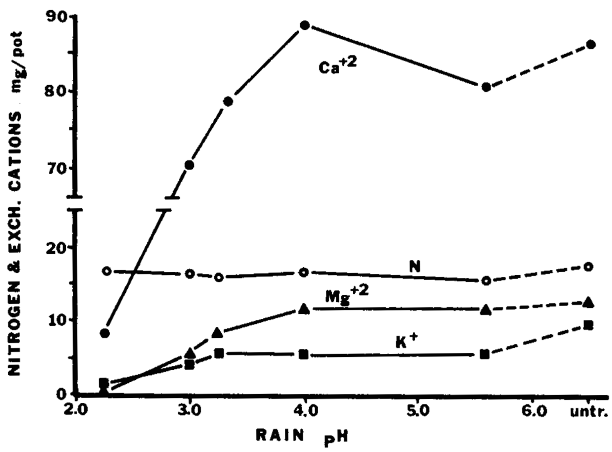

Figure 3c. Exchangeable cations and Total N before and after the experimental period. (Untr = Untreated soil).

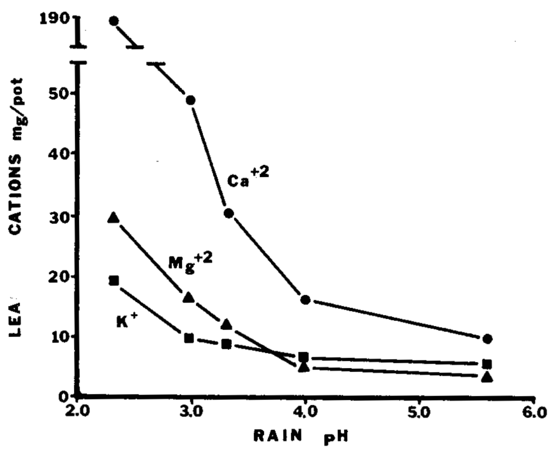

Figure 3d. Estimated total leaching losses of K^{+1}, Mg^{+2}, and Ca^{+2} after 20 treatments.

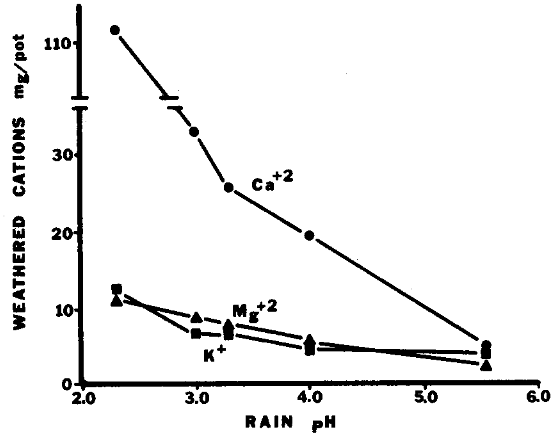

Figure 3e. Estimated weathering inputs of K^{+1}, Mg^{+2}, and Ca^{+2} over the 20-week experimental period.

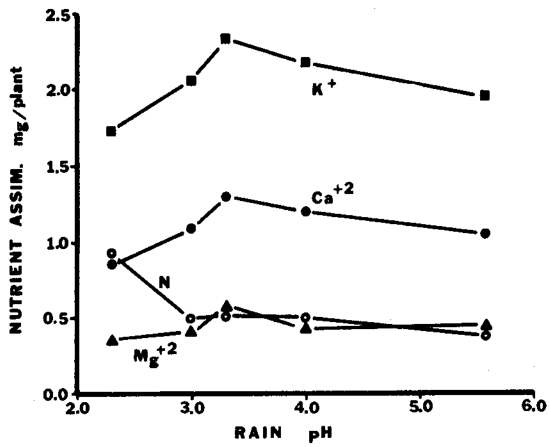

Figure 3f. Seedling assimilation of K^{+1}, Mg^{+2}, Ca^{+2}, and N measured at the end of the experimental period.

Although we did not employ titration to precisely measure H^+ exchange processes within the soil, our pH determinations of "rain", soil, and leachate (Fig. 3b) suggest the importance of this mechanism. pH differences between "rainwater" and soil leachate indicate uptake of H^+ by the soil via cation exchange and/or neutralization of acids by basic compounds in the soil occurring at all treatment levels, but increasing with "rain" acidity. Declines in soil pH provide further evidence for H^+ uptake via exchange.

Mean leaching losses of K^{+1}, Mg^{+2}, and Ca^{+2} continuously rose with rain acidity (Fig. 3d). T-tests indicate that for Mg^{+2} and Ca^{+2}, leaching losses significantly increased with each increase in the H^+ concentration of the rain. Significant increases in the leaching of K^{+1} were observed at pH's of 3.0 and below. Two mechanisms involving hydrogen ions may underlie these trends: 1) H^+ exchange with cations held on soil binding sites; and 2) Acid induced weathering of soil minerals.

Measurments of exchangeable cations retained by the soil following the 20 treatments (Fig. 3c) suggest that at pH's of 4.0 and above, losses via leaching and plant uptake were balanced by weathering inputs. No continuous declines below values for untreated soil are evident in this range. At pH 3.3, decreases in exchangeable Mg^{+2} occurred. At pH 3.0, exchangeable K^{+1}, Mg^{+2}, and Ca^{+2} decreased to between 54% and 84% of their respective mean concentrations at higher pH's, and at pH 2.3, exchangeable cation concentrations decreased to very low levels. Depletions of available cation reserves may have resulted from saturation of exchange sites with H^+ and subsequent decrease in the ability of the soil to hold nutrient cations and/or a situation of leaching outputs outpacing weathering inputs. Soil Kjeldahl nitrogen appeared to be unaffected by rain acidity. Soil nitrate-nitrogen levels were undetectible (less than 0.01 μg/g soil) in all treatments. The water-soluble nitrate was apparently rapidly leached from the pots by the "rain".

Estimated weathering inputs of K^{+1} and Mg^{+2} rose continuously but linearly with logarithmic increases in the acidity of the simulated rain (Fig. 3e). Three to four-fold increases coincided with 1500-fold increases in "rain" acidity (pH 5.6 to pH 2.3). Weathering of Ca^{+2} responded more logarithmically with an approximate 22-fold increase over that pH range.

The greatly accelerating weathering rates estimated for Ca^{+2} raise several questions. How long might supplies of weatherable Ca^{+2} last? Were these reserves seriously depleted at pH 2.3 over the 20 week period? We made no direct measures to answer these questions. However, the steady declines in the leaching losses of Ca^{+2} from Week 5 through Week 19 (Fig. 4) may suggest concurrent declines in Ca^{+2} weathering,

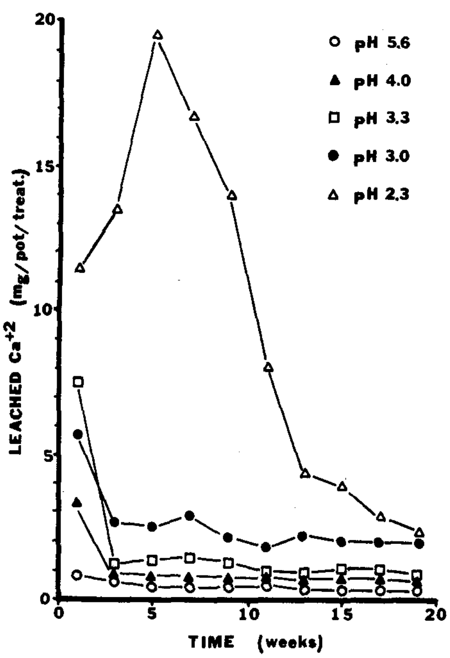

Figure 4. Weekly leaching losses of Ca^{++} through the 20-week experimental period.

and accordingly, decreases in the supplies of weatherable Ca^{+2}. (Similar temporal patterns were observed for K^{+}, and Mg^{+2} leaching.) It must be stressed that this evidence in only circumstantial. Declines in exchangeable Ca^{+2} and in the base saturation of the soil measured at pH 2.3 might also promote the pattern of decreasing Ca^{+2} leaching.

Nutrient assimilation by the pine seedlings showed some relationship to precipitation chemistry. Organic nitrogen levels in the plant tissue (lowest at pH 5.6 and highest at pH 2.3) probably reflect NO_3^{-1} levels in the rainwater. Amounts of assimilated K^{+1}, Mg^{+2}, and Ca^{+2} vary with plant weight through the pH range 5.6 - 3.0, indicating fairly uniform concentration in plant tissue. At pH 2.3, however, decreased amounts of assimilated cations resulted in spite of increased plant weight. Stem and leaf concentrations of the three measured cations dropped to between 1/2 and 2/3 of concentrations at lower acidity levels. These declines may be results of the low levels of available cations in the pH 2.3 soils, and/or major increases in foliar leaching expected at this treatment level (10).

An over-all effect of our pH 2.3 acid "rain" was to produce, after 20 weeks, an acid soil, quite low in supplies of exchangeable cations and, perhaps, weatherable cations. Declines in seedling assimilation of these nutrients at pH 2.3 resulted. Despite these trends, seedling productivity increased with rain acidity during the 20-week period. High nitrate inputs through rainwater were probably responsible. Nitrogen, however, can only stimulate growth when adequate supplies of all essential nutrients are present. The low levels of available K^{+1}, Mg^{+2}, and Ca^{+2} at pH 2.3 apparently did not become limiting during the 20-week treatment period, but questions are raised concerning how much longer decreasing cation supplies may have supported growth. We feel that the increased productivity of the pine seedlings noted at pH 2.3 may have been a short-term phenomenon.

SUMMARY AND CONCLUSIONS

1. 125 Eastern White Pine seedlings, individually potted in sandy loam soil, were randomly divided into five treatment groups of 25 plants. Each group was subjected to 20 weekly treatments with a simulated acid rain adjusted to one of the following pH's: pH 5.6, pH 4.0, pH 3.3, pH 3.0, and pH 2.3. "Rains" were solutions of sulfuric, nitric and hydrochloric acids. Soil leachate was collected during treateents and subsequently analyzed. Soil chemistry, seedling tissue chemistry, and seedling productivity were measured at the end of the 20-week period.

2. Monitoring of "rain", soil, and leachate pH's indicated uptake of H^{+} by the soil, probably through ion exchange mechanisms. Marked soil pH declines were measured after 20 weeks of treatment at pH 2.3.

3. Leaching of Mg^{+2} and Ca^{+2} steadily increased with "rain" acidity through the pH range 5.6 - 2.3. K^{+1} losses did not increase until the "rain" pH was lowered to 3.0 and 2.3.

4. Declines in exchangeable K^{+1}, Mg^{+2}, and Ca^{+2} were measured at pH's of 3.0 and below. Greatly depleted levels of exchangeable cations were found at pH 2.3.

5. Estimated weathering inputs of K^{+1}, Mg^{+2}, and Ca^{+2} steadily increased with "rain" acidity through the pH range 5.6 - 2.3. Ca^{+2} inputs rose most dramatically with a 22-fold increase over that pH range.

6. Declines in cation leaching (at pH 2.3) through time suggest that supplies of weatherable K^{+1}, Mg^{+2}, and Ca^{+2} may have been greatly reduced by the 20 pH 2.3 treatments.

7. K^{+1}, Mg^{+2}, and Ca^{+2} assimilation by the seedlings was fairly constant through the pH range 5.6 - 3.0. Declines were observed at pH 2.3. Nitrogen assimilation, fairly constant from pH 5.6 to 3.0, rose significantly at pH 2.3, probably as a result of high NO_3^{-1} inputs with rainwater.

8. Seedling productivity increased significantly with rainwater acidity. Lowest growth was measured at pH 5.6, and the greatest growth at pH 2.3. NO_3^{-1} fertilization probably caused this trend.

9. Nitrogen can only stimulate growth when supplies of available cations are adequate. Increased leaching losses of K^{+1}, Mg^{+2}, and Ca^{+2} and the declines in exchangeable (and perhaps weatherable) cations measured at pH 2.3 raise questions regarding how long declining supplies of available cations might support growth. The accelerated productivity noted at pH 2.3 may be a short-term phenomenon.

10. Foliar damage, observed only at pH 2.3, was not associated with declines in seedling productivity over the 20-week period. Questions are raised regarding the impact of needle injury on long-term growth.

REFERENCES

Dahl, E. and O. Skre. 1971. An investigation of the effect of acid precipitation on land productivity. *In* Konferens om avsvavling, Stockholm. 11 Nov. 1969. NORDFORSK, MILJÖVARDSSKRETARIATET, pub. 1971. 1: 27-29.

Gordon, C. C. 1972. Short-long conifer needle syndrome. Interim report to the Environmental Protection Agency. Mimeo.

3. Jonsson, B., and R. Sundberg. 1972. Has the acidification by atmospheric pollution caused a growth reduction in Swedish forests? A comparison of growth between regions with different soil properties. *In* B. Bolin (ed.), Supporting studies to air pollution accross national boundaries. The impact on the environment of sulfur in air and precipitation. Sweden's case study for the United Nations Conference on the Human Environment. Royal Ministry of Foreign Affairs. Royal Ministry of Agriculture, Stockholm.

4. Mecklenburg, R. A., H. B. Tukey, Jr. and J. V. Morgan. 1966. A mechanism for the leaching of calcium from foliage. PLANT PHYSIOLOGY. 41: 610-613.

5. Odén, S., R. Andersson and M. Bartling. 1972. The longterm changes in the chemistry of soils in Scandanavia due to acid precipitation. *In* B. Bolin (ed.), Supporting studies to air pollution across national boundaries. The impact on the environment of sulfur in air and precipitation. Sweden's case study for the United Nations Conference on the Human Environment. Royal Ministry of Foreign Affairs. Royal Ministry of Agriculture, Stockholm.

6. Overrein, L. N. 1972. Sulfur pollution patterns observed; leaching of calcium in forest soil determined. AMBIO. 1: 145-147.

7. Whittaker, R. H., F. H. Bormann, G. E. Likens, and T. G. Siccama. 1974. The Hubbard Brook Ecosystem Study, forest biomass and production, ECOL. MONOG. 44: 233-252.

8. Wilde, S. A., G. K. Voigt, and J. G. Iyer. 1972. Soil and plant analysis for tree culture. Oxford and IBH Publishing Co. New Delhi.

9. Wood, T. and F. H. Bormann. 1974. The effects of an artificial acid mist upon the growth of *Betula alleghaniensis*, BRITT. ENVIRON. POLLUT. 7: 259-268.

10. Wood, T. and F. H. Bormann. Increases in foliar leaching caused by acidification of an artificial mist. Submitted to Ambio.