# INVESTIGATION OF THE ACIDITY OF LEACHATES FROM DECOMPOSING LEAVES OF DECIDUOUS TREES

A Thesis

by

Graeme W. Boswall

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# INVESTIGATION OF THE ACIDITY OF LEACHATES FROM DECOMPOSING LEAVES OF DECIDUOUS TREES

## I INTRODUCTION

Podsol soils constitute a large proportion of the soils of Eastern Canada (1). They are developed under woodland in cool humid conditions where rainfall exceeds evaporation. The profile from the surface downward consists essentially of an organic layer, a leached layer, and a layer of accumulation just above the parent rock. Thus the simplest podsol profile consists of three horizons, namely: $A_0$, the raw humus layer; $A_2$, the leached siliceous layer; B, the layer of accumulation of sesquioxides.

The $A_0$ horizon of acid peaty organic matter is considered essential to the development of a true podsol. G.W. Robinson (2) makes the following statement:

> "...... It would appear that an acid reaction is not in itself sufficient to cause podsolization. This may be readily shown in the laboratory by allowing 0.1 N solutions of hydrochloric acid and oxalic acid, respectively, to percolate through ferruginous sand. Alghough the pH of the hydrochloric acid is lower than that of the oxalic acid, the solvent action of the latter acid is considerably greater and quickly leads to a bleaching by removal of ferric oxide......"

H.T. Jones and J.S. Willcox (3) have demonstrated in the laboratory that oxalic and tartaric acids will bring sesquioxides, from the upper part of the profile, into solution and deposit them lower down. S.U. Pickering (4) had shown earlier that ferric oxalate is a normal salt, but that so-called ferric tartrate, malate, and citrate are ferric compounds closely resembling the cupri compounds. The iron is in the electronegative ion and does not give the usual reactions of iron. These compounds are very soluble. In addition he has shown that concentrated solutions of ferric hydroxide, in hydroxy-acids, precipitate iron on standing. Thus Jones and Willcox concluded that hydroxy-acids play a part in the solution of sesquioxides, which are translocated in combination as complex anions, and precipitated as basic salts.

These organic acids have not been shown to be present in podsol soils, but investigations of the acidity of podsols have been made at Macdonald College, Quebec. H.J. Atkinson and R.R. McKibbon (5) made a study of the acidic nature of the water soluble substances, in the "raw humus" of the $A_0$ horizon of podsol soils of Quebec. The "raw humus" was leached in percolators with distilled water. These leachates were concentrated by evaporation on a steam bath. The amount of orginial acidity that remained in the concentrate varied from 83.5 to 99.1 per cent. Sulphuric and phosphoric acids were determined on the dialysable fractions of these concentrates. Total sulphuric acid was found in amounts varying from 30 to 52 per cent of the total acidity, while free sulphuric acid amounted to 9.5 to 23 per cent of the total acidity of the percolate. Phosphoric acid was found to be present in amounts varying from mere traces to as much as 28 per cent of the total acidity, if it were present in the free state.

Later work along these lines was reported by Sallans _et al_ (6). Mannitol was obtained in amounts of 3 to 4 per cent of the dissolved organic matter and free acetic acid was found to be present in an amount equivalent to about 8 per cent of the total acidity. There was also evidence of the presence of formic acid in the free state.

Shorey (7) isolated several acids, including oxalic and succinic acids, from soil, but the methods used make it appear highly improbable that these acids occurred in the free state.

The present investigation has been carried out on the leachates of decomposing leaves as distinct from the humus layer on the surface of the soil. The acidic nature of these leachates has been demonstrated in this laboratory (8), and a more detailed study of the nature of this acidity has been considered advisable.

Recently chromatographic methods have been developed for the separation, identification, and quantitative estimation of mixtures of organic acids of low molecular weight. Martin and Synge (9) first developed silica gel partition chromatography while working on protein hydrolysates.

Many of the acetyl derivatives of amino acids showed considerable differences in their partition coefficients between chloroform and water. Martin and Synge (10) constructed a counter-current extraction machine suitable for the chloroform-water system, but they found that this machine which had an efficiency of less than forty theoretical plates was inadequate to obtain clear cut fractions. They abandoned the idea of obtaining flow in both directions and an attempt was made to hold one phase stationary while the other was allowed to flow past it. Martin and Synge (9) found that silica gel would hold about half its weight of water, and consequently it was chosen to hold the water stationary while chloroform flowed past it. Thus a "partition chromatogram" was prepared with silica gel containing water, and using chloroform as the mobile phase, to effect a separation between various acetyl amino acids, making use of their differences in partition coefficients between chloroform and water. The bands of amino acids were revealed by methyl orange which was contained in the water phase.

Martin and Synge (9) also developed a general theory of chromatography applicable to substances with linear distribution isotherms. This theory expressed the efficiency of the chromatogram in terms of the number of theoretical plates to which it was equivalent, and for an average column it was found to be in the order of thousands.

In the case of a particular column, the partition coefficient deduced from the rate of movement of the bands agreed very closely with that determined directly. This was taken to show that, under favorable conditions, adsorption on the silica gel could be practically eliminated, and that the water in the silica had the same solvent properties as normal water.

E.L. Smith (11) demonstrated that the silica gel partition chromatogram developed by Martin and Synge (9) would separate mixture of formic, acetic, propionic, n-butyric, and n-valeric acids in chloroform into its component acids. S.R. Elsden (12) investigated the method further and developed a method for the separation, identification, and quantitive estimation of acetic, propionic, and butyric acids in a mixture containing all three acids.

Ramsey and Patterson (13) made use of a chromatographic partition column for the separation and identification of micro amounts of the saturated volatile fatty acids ($C_1$-$C_4$). Formic, acetic, and propionic acids were separated completely from one another, but n-butyric and iso-butyric acids were obtained together. Later they presented a method (14), based on partition chromatography for the separation and determination of the saturated straight-chain fatty acids $C_5$ to $C_{10}$. These fatty acids were separated on a column of silicic acid, using methanol as the immobile solvent, 2,2,4-trimethylpentane as the mobile solvent, and bromcresol green as the indicator.

Claborn and Patterson (15) found partition chromatography very useful in the determination of succinic acid in foods.

F.A. Isherwood (16) evolved a method which will separate quantitatively many of the acids commonly found in fruits, and if necessary, can be modified readily so as to allow the constituent acids to be isolated in a relatively pure state and identified. Briefly, the essential steps in his procedure are the extraction of the acids from an acidified extract of fruit by 50 per cent (v/v) n-butanol-chloroform, the preparation of a concentrated solution of the acids in 50 per cent (v/v) tert-amyl alcohol-chloroform and their final separation and analysis using a modified partition chromatogram.

The changes which Isherwood made in the chromatogram were (1) the aqueous solution of indicator was replaced by 0.5 N sulphuric acid. The 0.5 N sulphuric acid is superior to water as it depresses the ionization of acids such as oxalic and fumaric which would otherwise give very wide bands owing to the rapid increase in the partition coefficient with dilution. (2) The bands were revealed by continuously feeding an aqueous solution of an indicator into the solvent emerging from the bottom of the column.

Partition chromatography on paper also has been found to be a very useful method for the qualitative analysis of small amounts of various compounds. Martin et al (17) failed to separate the slower moving acetyl amino acids on a silica column, but later (18) they found that by substituting cellulose for silica more promising results were obtained. Following further work along these lines, Consden, Gordon, and Martin (19) described a micro-analytical method of separating amino acids on filter paper partition chromatograms. Water in the cellulose (filter paper) acts as the stationary phase of the chromatogram while phenol, collidine, and n-butanol-benzyl alcohol (1:1 v/v) were useful as mobile phases.

In making an analysis by the method of Consden, Gordon, and Martin, a small volume of the solution of amino acids (0.2 ml.) is placed on a paper strip which is hung from a trough containing the mobile solvent. The latter travels down the paper strip and separates the mixture of amino acids. The series of amino acid spots along the paper is revealed by spraying the dried paper with a solution of ninhydrin. The acids then can be identified by their $R_f$ values, that is the ratios of the distance the acids move to the distance the solvent front moves from the spot where the solution of acids was placed. When using only 200 ug. of wool, Consden et al (19) were able to demonstrate by this method the presence of all the amino acids which have been shown to be there by other methods.

Partridge (20) adopted the method for the separation of reducing sugars, the spots being revealed by spraying the paper with a solution of ammoniacal silver nitrate. Flood, Hirst, and Jones (21) modified this method for the quantitative determination of the separated sugars.

Lugg and Overell (22) adapted the method of Consden <u>et al</u> (19) to the separation and determination of carboxylic acids. They found that a mixture of 20 ug. each of malic, citric, and tartaric acids were resolved with ease. The mobile solvent was n-butanol which was saturated with acetic acid to depress the ionization of the carboxylic acids, and thus prevent tailing of the spots.

Both silica gel and paper chromatography have been applied in the present investigation, the object of which was to isolate and identify some of the low molecular weight organic acids that might be present in the leachates.

## II EXPERIMENTAL MATERIALS

### 1. Leaves

Leaves from four species of trees were used in this investigation, namely: beech (Fagus gradifolia), birch (Betula populifolia), maple (Acer saccharum), and poplar (Populus tremuloides and Populus grandidentata).

In September, 1947, sites were selected in The Morgan Arboretum at Macdonald College, Quebec, for the purpose of collecting and obtaining an estimate of the leaf fall from the four species of trees. In the areas selected, the undergrowth and partly decomposed litter were cleared away, care being taken not to disturb the surface soil. In the case of the birch stand, which was located in a pasture, the sward was carefully raked to remove dead grass, leaves, and twigs.

The prepared sites were then enclosed with rot-proofed canvas windscreens. Two sites were prepared under each species. Table I records the area at each location and the total area enclosed by windscreens under each species.

The leaves were collected by picking them up by hand at frequent intervals shortly after they began to fall. Only in one or two instances were the fallen leaves exposed to rain before being collected. Leaves of other species which fell inside the screened area were discarded. The birch leaves collected were contaminated with a small

TABLE I

AREA UNDER EACH SPECIES ENCLOSED BY WINDSCREENS

| Species | Site No. | Area (sq.ft.) | Total Area (sq.ft.) |
|---|---|---|---|
| Beech | 1 | 113 | |
| " | 2 | 113 | 226 |
| Birch | 1 | 468 | |
| " | 2 | 284 | 752 |
| Maple | 1 | 113 | |
| " | 2 | 113 | 226 |
| Poplar | 1 | 113 | |
| " | 2 | 113 | 226 |

amount of grass as they were raked instead of hand-picked.

The leaves of each species were put in separate bags as collected, weighed, and stored in a small building in The Morgan Arboretum. Representative samples of each collection were taken into the laboratory. The data are summarized in Table II.

The percentage dry matter, oven-dry weight of leaves collected, and leaf fall in pounds per acre based on oven-dry weight are given in Table III.

## 2. Leachates

After a considerable quantity of leaves had been collected, those of each species were mixed thoroughly and

## TABLE II

## WEIGHT OF LEAVES COLLECTED (GRAMS)

| Date Collected (1947) | Beech | Birch | Maple | Poplar |
|---|---|---|---|---|
| Oct. 9 | 385 | 3100 | | 460 |
| " 13 | 1950 | | 400 | |
| " 14 | | 3585 | | 1320 |
| " 18 | 1105 | | 1310 | 1770 |
| " 20 | 1600 | | | |
| " 21 | | | 3105 | 1255 |
| " 22 | | 2950 | | |
| " 25 | | | 1995 | 270 |
| " 28 | | | | 190 |
| " 31 | 725 | | 1090 | |
| Nov. 4 | | 2845 | 130 | 95 |
| Total weight as collected | 5765 | 12480 | 8030 | 5360 |
| Total air dry weight | 4364 | 9887 | 7041 | 3680 |

## TABLE III

## PERCENTAGE DRY MATTER CONTENT OF LEAVES

| Species | Percent Dry Matter Determined on Air Dried Leaves | Total Oven-Dry Weight of Leaves Collected (G.) | Leaf Fall(lbs/A) Calculated on $H_2O$-Free Basis |
|---|---|---|---|
| Beech | 91.3 | 3984 | 1691 |
| Birch | 90.4 | 8938 | 1141 |
| Maple | 90.2 | 6351 | 2693 |
| Poplar | 91.1 | 3352 | 1421 |

placed on 1/8" mesh wire screens, supported on trays specially designed to collect the natural rainfall percolating through the layer of leaves. Each tray was 4' x 3' x 10" with a bottom sloping towards the centre and front terminating in a pipe connected to a covered pail. The trays were set into the ground with the top about 1" above ground level. A trench was dug the required depth to hold the pails for collecting the leachates resulting from natural rainfall. The trays were covered with ¼" mesh wire screen to exclude small animals, wind-blown leaves, etc. All surfaces of screens, trays, and pails were treated with asphalt paint to inhibit corrosion.

The dates of collection and weight of leaves put in each tray are recorded in Table IV.

The leachates were brought into the laboratory, immediately filtered to remove any solid particles present, and the volumes were recorded. Some routine determinations such as pH, titratable acidity, etc. were made on aliquots and the remainder of the leachates were preserved for study at a later date. Most of the colored material in the remaining leachates was precipitated by addition of a 0.5 M solution of lead acetate or was concentrated in small volume by distilling off the water at low temperature in vacuo.

## TABLE IV

## WEIGHT OF LEAVES IN TRAYS (GRAMS)

| Date (1947) | Species | Weight of Leaves in Trays<br>Air-Dry Weight | <br>Oven-Dry Weight |
|---|---|---|---|
| Oct. 20 | Beech | 2095 | |
| " 22 | " | 1430 | 3218 |
| " 20 | Birch | 4106 | |
| " 22 | " | 2599 | 6046 |
| " 20 | Maple | 1245 | |
| " 22 | " | 2730 | 3585 |
| " 20 | Poplar | 2154 | |
| " 22 | " | 928 | |
| " 28 | " | 208 | 2997 |

# III EXPERIMENTAL METHODS

Each leachate collected was characterized by determination of pH, titratable acidity or alkalinity, and total ash. The pH of the leachates was determined using a Beckman, Model G. potentiometer with glass electrode, immediately after the leachates were brought to the laboratory and filtered. The titratable acidity or alkalinity was determined by pipetting a 100 ml. aliquot of the leachate into an Erlenmeyer flask. The leachate was titrated with 0.01 N sodium hydroxide or hydrochloric acid to pH 7.0. The ash content was estimated by using a tared platinum evaporating dish to which was added 100 ml. of leachate. The solution was evaporated to dryness on a steam bath and then transferred to an electric muffle furnace of 550°C for one hour. The platinum dish and contents were allowed to cool in a desiccator and finally weighed.

## 1. Concentration of Materials in the Leachates

The leachates contained very small amounts of dissolved or colloidal material. During the period of the investigation the total solids in the leachates varied from 0.01 per cent in beech leachates to 0.17 per cent in birch leachates. In order to investigate the acidic compounds present it was decided either to separate the acidic compounds by precipitation or to concentrate the leachates. Both these methods were employed.

Experiment showed that a solution of neutral lead acetate precipitated most of the colored material in the leachates as a reddish-brown gelatinous mass. When dried this precipitate could be stored in tightly stoppered bottles until studies could be made on them. In this way it was hoped to obtain a material which would not undergo any change during storage. In addition a concentration of the leached material was accomplished.

It was found that 20 ml. of 0.5 M solution of neutral lead acetate per liter of leachate was sufficient to give maximum precipitation. After standing for a time the supernatant liquid was siphoned off, the residue centrifuged, and the remaining supernatant liquid was readily poured off. The gelatinous precipitates were then frozen and on thawing the precipitate was thrown out of solution as dark brown particles. The supernatant liquid was decanted off and the precipitate filtered and washed with cold distilled water on a Buchner funnel. The precipitate was finally dried under reduced pressure at room temperature.

Distillation under reduced pressure was also employed, the object being to obtain the non-volatile acidic materials in concentrated solution free from lead. With a view to carrying out these distillations as efficiently and as rapidly as possible, four sets of distillation apparatus (one for each species of leachate) were set up. Each apparatus consisted of a 3-liter flask connected to two condensers in series. Two condensers were used as it was found that when only one condenser was present, there was a portion of the distillate not condensing, and it was passing out of the receiving flask into the evacuating system.

Each distillation apparatus was connected to a short length of suction tubing, via a stopcock, which in turn was connected to the main suction tube from the vacuum pump. Capillary tubes were inserted in each distillation flask to prevent bumping.

To prevent the entrance of vapors into the vacuum pump, it was necessary to pass the vapors, which were being drawn out of the receiving flask by the pump, through first a desiccator containing sulphuric acid, and secondly through a round bottomed flask containing concentrated sulphuric acid through which bubbled the evacuated air.

The average vacuum obtained was about 70 cm. of mercury and the leachates distilled at a temperature seldom exceeding 50°C. The distillation was continued until only a small volume of leachate remained in the distillation flask.

In order to separate the acidic from non-acidic materials of the concentrates the latter were electrodialysed. The electrodialysis apparatus (Fig. 1) consists of five compartments or cells. The material to be electrodialysed was put in the centre cell in which a mechanical stirrer was operated to prevent any solid material from settling to the bottom. The two cells on either side of the centre cell were filled with distilled water, as were the outside compartments containing the platinum electrodes. Membranes of vegetable parchment were inserted between the centre and cathodic cells and cellophane membranes were inserted between the centre and anodic cells. The electrodes were connected to a 220 V. D.C. circuit and during electrodialysis, dialysable acidic materials moved into the anodic (+) cell and dialysable basic materials moved into the cathodic (-) cell. Cold water was passed over the outside of the cells during electrodialysis to keep the temperature as low as possible.

## 2. Silica Gel Partition Chromatography

Several workers (16, 23, 24) have experienced difficulty in obtaining a satisfactory silica gel from water glass. Isherwood (16) found that the presence of impurities such as $Fe^{+++}$ and $Al^{+++}$ causes the silica to have adsorptive properties and also that the structure of the gel is connected with adsorption. Adsorption must be reduced to a minimum in order to get a satisfactory recovery of acids present. He radically changed the method of preparation by making use of 10 N hydrochloric acid for the removal of impurities. The washed gel was then dried

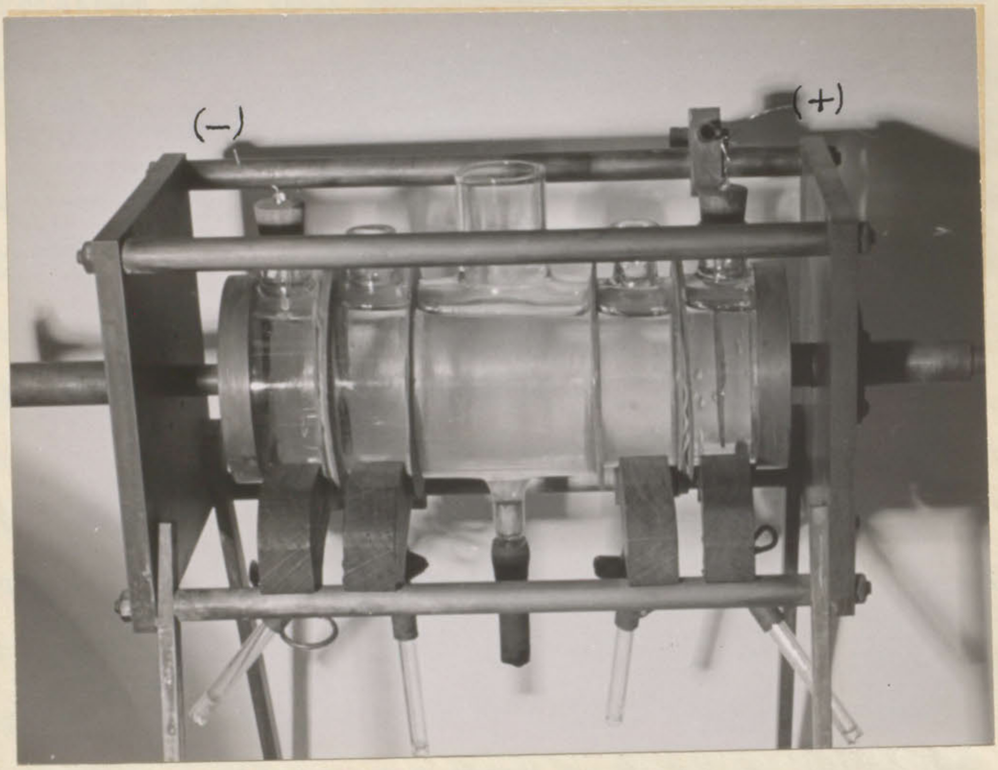

Fig. 1. Electrodialysis Apparatus

with ethanol and ether and allowed to stand for twenty-one days when the treatment with hydrochloric acid was repeated. The resulting gel, after standing over phosphorus pentoxide, is capable of adsorbing its own weight of water and still appears superficially dry.

Tristram (23) when working with amino acids, noted the extreme acidity of the silica gel prepared by Isherwood; nevertheless the recovery of amino acids was satisfactory. In this present work the extremely acid nature of silica gel prepared by Isherwood's method was encountered and it caused the gel to be unsatisfactory when employing an external indicator to reveal the organic acids in the eluate. Distilled water to which was added some silica gel had a pH of 1.65, and evidently the gel imparted this acidity to the chloroform-n-butanol solvent as it passed through the column. The indicator gave an acid reaction even when organic acids were absent. A more alkaline indicator was shown to be of no value, and at the same time if it should have shown promise, it was felt that its sensitivity might be greatly decreased. Therefore, it was necessary to prepare a less acidic silica gel. This was accomplished by the use of sodium hydroxide in the final part of the preparation of the gel.

The preparation of a satisfactory gel was carried out in the following manner: one pound of commercial water glass was diluted with twice its volume of warm distilled water, and filtered twice through a hardened filter paper (Whatman No. 50) to remove a small quantity of dark colored suspended matter. A little methyl orange indicator was added followed by 10 N hydrochloric acid in a thin stream with vigorous stirring by a mechanical stirrer. A thick porridge often resulted and sufficient distilled water to enable continued stirring was added. The addition of 10 N hydrochloric acid was continued until the mixture became acid when a further 35-50 ml. of hydrochloric acid were added. Stirring was continued for several hours and then the gel was filtered on a Buchner funnel using Whatman No. 50 filter paper. When the residue was sucked as dry as possible, it was suspended in 850 ml. of 10 N hydrochloric acid and allowed to stand overnight at room temperature. It was again filtered almost dry but not allowed to crack, and the gel was washed with 1.8 l. of 5 N hydrochloric acid. Distilled water was then passed through the gel until the filtrate was free from chlorides. The gel was then washed with 1.8 l. of absolute ethanol and finally 1 l. of dry ether. The silica gel was dried at room temperature and allowed to stand for three weeks. It was then suspended in 1.2 l. of 10 N hydrochloric acid and allowed to stand overnight. It was again washed as before and suspended in distilled water; 0.5 N sodium hydroxide then was added until the suspension was about neutral in reaction. After standing overnight it was filtered, washed with distilled water, absolute ethanol, and finally with dry ether. The gel was dried at 160°C for three days and then placed in a tightly stoppered bottle.

Several batches of gel were prepared by this method and the gel from most of these batches was satisfactory for partition chromatograms. Distilled water to which was added some silica gel had a pH range of from 5.5 to 7.5. The silica gels which were found satisfactory were able to adsorb their own weight of 0.5 N sulphuric acid and still appeared superficially dry. The silica gels were sieved and the portion which passed through a 100-mesh sieve was used for the analysis of acids.

Batches of silica gel that would not adsorb their own weight of 0.5 N sulphuric acid were considered unsatisfactory for partition columns and were discarded. It was noted that if the silica gel was allowed to become dry and channel when filtering and washing, it would not dry as a fine powder, but would form into rather coarse granules. Only a small portion of these gels would pass through a 100-mesh sieve. This seems to indicate that the ethanol did not pass through the gel uniformly, but rather followed the channels that formed if the gel were allowed to dry out. Consequently, great care was taken to prevent channeling, and satisfactory gels were obtained thereafter.

One batch of silica gel was also prepared from sodium metasilicate (25). This gel was prepared in the following manner. The contents of a 1 lb. bottle of reagent grade sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$) were mixed with sufficient water to give a final volume of approximately 620 ml. This was heated and stirred on a steam bath until the silicate was dissolved. It was then cooled to about 30°C and some methyl orange indicator was added. Concentrated hydrochloric acid (C.P.) was then added in a thin stream with vigorous stiring until the mixture was permanently acid to methyl orange, when 10 ml. more were added. It was then allowed to stand for three hours, and more acid was added as the indicator changed to yellow. The silica gel was then filtered off on a large Buchner funnel and washed with 5 l. of distilled water. It was then resuspended in 0.2 N hydrochloric acid and allowed to stand overnight. The gel was filtered and washed with distilled water until no chlorides were present in the washings. Finally the gel was washed with 500 ml. of ethanol followed by 500 ml. of dry ether. It was dried at room temperature in paper boats, and finally heated at 160°C for three days. This silica gel also was too acidic for use in a partition chromatogram employing an external indicator, so it was suspended in distilled water and 0.5 N sodium hydroxide was added until the pH of the suspension was approximately 7.0. After standing for a few hours it was filtered and washed with distilled water, ethanol, and ether. It was dried at room temperature and then at 160°C for three days. Distilled water to which was added some of this gel had a pH of 6.25.

This gel was much different in composition from the gel prepared from water glass. It was passed through a 100-mesh sieve and although most of it passed through this sieve, the particle size appeared to be much larger on the average than the particles of gel prepared from water glass. In addition it was not capable of holding as high a percentage of water and still remain superficially dry.

The thymol blue indicator and the solvents, viz. 50 per cent (v/v) n-butanol-chloroform, 50 per cent (v/v) tert-amyl alcohol-chloroform, and 5, 10, 20 and 35 per cent n-butanol-chloroform used in silica gel chromatography were prepared according to Isherwood (16).

The partition chromatogram as modified by Isherwood was used for the analysis of only three known solutions and one acid solution obtained from a leaf leachate. At that time "filter paper partition chromatography" was developed in this laboratory for the identification of organic acids, some of which were shown to be present in the leachates. It was then found very convenient to collect successive 50 ml. portions of the solvent emerging from the column, and to subject these fractions to analysis by filter paper chromatography.

The apparatus used in the preliminary fractionation over silica gel can best be appreciated by referring to Fig. 2. The glass tube A is 75 cm. long and 1.3 cm. internal diameter. The tube has a constriction near the bottom upon which is resting a glass disc. On this disc is placed a single layer of filter paper slightly larger than the internal cross section of the tube. The glass tube is connected near the top to a pressure source, via stop-cock B. C is a 2-litre suction flask which is attached to a source of reduced pressure, via the stop-cock D. The glass tube E passes into the suction flask and extends to the bottom of a 50 ml. test tube F while the other end terminates in a 125 ml. Erlenmeyer flask G. The test tube F is suspended by a cord from the stopper of the suction flask. The flask G is also connected to a source of reduced pressure, via stop-cock H.

In operating the apparatus, the tube A is filled to the arm with solvent and then stoppered. Stop-cock I is closed, stop-cocks D and H are opened and the suction flask and Erlenmeyer flask become equally evacuated. Pressure is exerted on the top of the solvent by opening stop-cock B. The difference in pressure between the top and bottom of the partition column is about 2 atmospheres, which gives a flow of solvent of from 2-4 ml. per minute. When the solvent in the test tube F has reached the 50 ml.

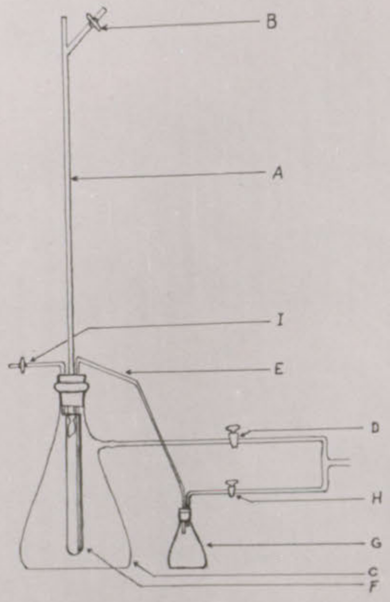

Fig. 2. Silica Gel Chromatogram Apparatus.

mark, stop-cock D is closed and I opened. The pressure difference between the suction flask and Erlenmeyer flask transfers the 50 ml. fraction to the Erlenmeyer flask. H is then closed and the Erlenmeyer flask is replaced by another to collect the next fraction. By closing I and opening D and H the system is again evacuated and another 50 ml. fraction is collected.

A solution containing six acids, namely: succinic, malonic, oxalic, malic, citric, and tartaric was subjected to chromatographic separation. These acids were selected as it was thought that organic acids of low molecular weight normally produced by micro-organisms might be found to occur among the leachable products of decomposing leaves.

The procedure followed in the separation of the acids was essentially the same as that given by Isherwood, but the following two modifications were found helpful. (1) The application of pressure to the solvent head to give a satisfactory rate of flow of the solvent. (2) After the silica gel was added to the glass tube, several ml. of solvent were passed through it to remove some excess acidity that would leach from the column. If this acidity were allowed to remain it would interfere in the detection of the organic acid bands when using an external indicator.

Consequently the procedure used is briefly as follows: 3.0 g. of silica gel were added to 3.0 ml. of 0.5 N sulphuric acid. 10 per cent n-butanol-chloroform was added to this mixture and the slurry was quantitatively transferred to the glass tube. Sufficient pressure was then applied to the head of the solvent to obtain a flow of 2-4 ml. per minute. The silica gel was allowed to pack, but care was taken that it did not dry out and channel. The indicator solution was adjusted to one-tenth the rate of flow of the solvent through the column, and solvent was passed through until it imparted a blue color to the indicator. The column was then allowed to run just dry and 2 ml. of 50 per cent tert-amyl alcohol-chloroform solution of the acids were added dropwise from a pipette so as not to disturb the silica gel. When this solution had drained into the gel, 1 ml. of the appropriate strength of the developing solvent (in this case 10 per cent n-butanol-chloroform) was added very carefully so as to wash any traces of the solution on the walls of the tube into the gel. This solvent was allowed to drain into the gel, and the process was repeated twice. The glass tube was then filled with the solvent and more solvent added as required. The indicator was run into the eluate at the rate of one tenth the corresponding volume of eluate. The color of the mixture of indicator and eluate flowing down the capillary stem of the funnel was closely observed. Isherwood states: "...... When an organic acid commences to wash out of the column, the color changes to green and then to yellow, the solvent becoming a distinct pink......" These changes were not very pronounced when using 5 and 10 per cent n-butanol-chloroform solvents and succinic or malonic acid was being washed out of the column, but the color change was sharp with solvents of higher n-butanol content when the rest of the acids were being separated. Consequently when using 5 and 10 per cent n-butanol-chloroform it was often difficult to know when an organic acid was beginning to wash from the column. When the color changed from blue to red the receiver was changed, and when all the organic acid was washed out the reverse color change occurred, and the receiver was changed as soon as the color was blue. This procedure was repeated each time an organic acid was washed out of the column. Proceeding in this way the mixture of acids originally added to the top of the column could be resolved into a series of fractions. After the first acid band left the column 20 per cent n-butanol-chloroform was used, and 35 per cent n-butanol-chloroform was used after the third acid band left the column. The volumes of the eluate and indicator were recorded. Each fraction was then titrated with 0.01 N barium hydroxide to pH 8.4 after 10 ml. of distilled water were added.

A small correction must be applied to the titration value due to the acidity of the solvent and the alkalinity of the indicator although these almost compensate for one another. Table V shows the titration blank per ml. of the solvent.

## TABLE V

## ACIDITY OF THE SOLVENTS

| Solvent | Volume of 0.01 N $Ba(OH)_2$ to Bring 1 ml. Solvent to pH 8.4 |
|---|---|
| 5 per cent n-butanol-chloroform | 0.010 ml. |
| 10 " " " | 0.012 " |
| 20 " " " | 0.015 " |
| 35 " " " | 0.020 " |

By titrating an aliquot of the indicator with 0.01 N hydrochloric acid it was found that 1 ml. of the indicator required 0.17 ml. of 0.01 N hydrochloric acid to lower its pH to 8.4. The titration value that is due to the organic acids in the fractions then becomes equal to:

Titration value - [(ml. solvent x a) - ( ml. indicator x b)]

where a = ml. of 0.01 N barium hydroxide per ml. of solvent

b = ml. of 0.01 N hydrochloric acid per ml. of indicator

The following formula was used for determining the weight of acid obtained in each fraction:

$$\text{mg.of acid} = \frac{\text{Mol.Wt. x ml.0.01 N } Ba(OH)_2 \text{x Normality x 1000}}{\text{1000 x No. of carboxyl groups}}$$

The first separation of a mixture of acids which showed any degree of success was made on a solution prepared by dissolving 30 mg. of total acids consisting of equal parts by weight of the acids mentioned in 2.0 ml. of 50 per cent tert-amyl alcohol-chloroform solvent. This separation was effected by using the external indicator method.

In Table VI the results are given where it is seen that only five acid fractions were obtained. The titration value of the first fraction indicated that two acids were present, and from their closely similar partition coefficients, both succinic and malonic acids would be expected in this fraction, while the other distinct fractions would contain successively oxalic, malic, citric, and tartaric acid. This later was confirmed by filter paper chromatographic analysis of the acid fractions from a similar known solution, the results of which are given in Table XIII.

TABLE VI

RECOVERY OF ACIDS FROM KNOWN SOLUTIONS (30 Mg.)

| Fraction | Solvent | Ml. of 0.01 N $Ba(OH)_2$(Corr.) | Acid | Per cent Recovery |
|---|---|---|---|---|
| 1 | 10 per cent | 16.00 | Succinic<br>Malonic | 88.5 |
| 2 | 20 per cent | 8.60 | Oxalic | 108.3 |
| 3 | 20 per cent | 6.39 | Malic | 85.7 |
| 4 | 35 per cent | 7.27 | Citric | 101.8 |
| 5 | 35 per cent | 6.49 | Tartaric | 97.4 |

## TABLE VII

RECOVERY OF ACIDS FROM KNOWN SOLUTIONS (60 Mg.)

| Fraction | Solvent | Ml. of 0.01 N $Ba(OH)_2$(Corr.) | Acid | Per cent Recovery |
|---|---|---|---|---|
| 1 | 10 per cent | 34.71 | Succinic<br>Malonic | 96.0 |
| 2 | 20 " | 14.91 | Oxalic | 93.9 |
| 3 | 20 " | 14.80 | Malic | 99.2 |
| 4 | 35 " | 14.71 | Citric | 99.3 |
| 5 | 35 " | 12.04 | Tartaric | 90.4 |

Table VII gives the results of a separation of the same acids, obtained from a mixture of 60 mg. of acids (i.e. 10 mg. of each acid). Again the first fraction contained two acids. The percentage recovery was more uniform than in the previous run and the five fractions obtained were quite distinct. This method gave promise of being capable of separating minute amounts of acids if present in the leachates.

A partition chromatogram of the silica gel made from sodium metasilicate was prepared, and the separation of the same six acids was attempted. This silica gel did not appear very promising when preparing the column. The mixture of 0.5 N sulphuric acid and silica gel did not give a superficially dry powder, but consisted more or less of a paste and when the n-butanol-chloroform solvent was added the gel formed into small masses or blobs about the size of peas. However, when added to the glass tube the gel packed quite readily and solvent passed through it at about the same rate as through gel prepared from water glass.

The results of the separation of acids are given in Table VIII. It is seen that this chromatogram, while giving three acid bands similar to those given by the water-glass silica gel, suddenly released an acid band (fraction 4) whose titration value indicates that most of the 3 ml. of 0.5 N sulphuric acid, which had been added to the gel, was present in the fraction. Since the gel prepared from water-glass gave satisfactory results, this sodium metasilicate gel was not studied further.

TABLE VIII

RECOVERY OF ACIDS FROM KNOWN SOLUTIONS (60 Mg.)

| Fraction | Solvent | Ml. of 0.01 N $Ba(OH)_2$(Corr.) | Acid | Per cent Recovery |
|---|---|---|---|---|
| 1 | 5% n-butanol-chloroform | 35.34 | Succinic<br>Malonic | 97.8 |
| 2 | 10% " | 13.38 | Oxalic | 84.3 |
| 3 | 20% " | 14.82 | Malic | 99.4 |
| 4 | 35% " | 151.56 | - | - |
| 5 | 35% " | 4.51 | - | - |

## 3. Filter Paper Partition Chromatography

In this present work investigations were carried out to find the optimum conditions necessary for satisfactory filter paper chromatographic separations of organic acids. Acetic acid-n-butanol solvent was first employed but the results were disappointing. Following this formic acid-n-butanol solvent was studied and in addition the capillary ascent technique as described by Williams and Kirby (26) was adopted. The results described in the following paragraphs are similar to those obtained by Lugg and Overell (27) in which the descent technique was used. However, their paper was not received until after this work was completed.

The objective was to develop a method, for the separation and identification of the low molecular weight organic acids on filter paper, to be used as a means of identifying the acids in the fractions obtained from the silica gel chromatograms. Filter paper partition chromatography seemed to be the most feasible method for identifying these acid fractions since very minute amounts of organic acids might be present in the leachates.

At first the separation of succinic, malonic, oxalic, malic, citric, and tartaric acids on filter paper was studied. When satisfactory separations of these acids were obtained other acids were included. Using known solutions of acids the primary problems investigated were:

(1) The volume of acid solution to be applied to the paper.

(2) The concentration of acid for the chosen volume. (3) Any changes, such as in $R_f$ value, with change in acid concentration. (4) The concentration of formic acid to be used in the solvent mixture. (5) Solvents other than n-butanol which could be used advantageously for "two-dimensional" chromatograms. (6) Determination of the partition coefficients of some of the known acids.

The apparatus consisted of a series of large glass bottles with screw covers which were well greased with stop-cock grease making the seal air-tight. In these bottles was placed about one quarter of an inch of a suitable solvent.

Sheets of filter paper 7.0 in. x 10.5 in. were cut from Whatman No. 1 filter paper sheets. The sheet so cut was formed into a cylinder which fitted into the glass bottle without coming in contact with the sides of the bottle. The edges of the filter paper were so sewn with cotton thread that they did not quite touch each other.

Solutions of the acids were spotted by means of a micropipette about 1 in. from the bottom of a filter paper cylinder and at intervals of $\frac{3}{4}$ in. These spots were allowed to dry at room temperature, and the cylinder was then placed vertically into the bottle. The end, at which were spotted the acid solutions, was placed into the solvent mixture. It was found that the acid spots obtained were better defined when the atmosphere within the bottle was allowed to become saturated with the solvent mixture before putting the filter paper cylinder into the bottle.

The apparatus was then made air-tight and the solvent was allowed to run up the filter paper to within about 1 in. of the top of the paper. This took about eight hours at the end of which time the cylinder was removed and allowed to dry at room temperature for about one day. The paper was then sprayed with indicator made by dissolving 40 mg. of bromcresol green in 95 ml. of ethanol and 5 ml. of distilled water. The indicator was adjusted to a blue color (pH 7.5) with 0.1 N sodium hydroxide. The acids were revealed as yellow spots against a blue background. The acids in a mixture were then identified by their $R_f$ values, which had been obtained separately by running a chromatogram in which single acids were applied in the spots (Fig. 3).

In Table IX are recorded the $R_f$ values of the different acids when applied to the filter paper in various concentrations. It is readily apparent from the data that the acids, when applied in a volume of either 0.01 ml. or 0.02 ml. of solution and chromatogramed, give suitably good spots on spraying with indicator. A volume of 0.01 ml. is probably slightly better than 0.02 ml. since the acid spot produced on spraying is smaller and more intense in color. All these acids give good spots when the acid

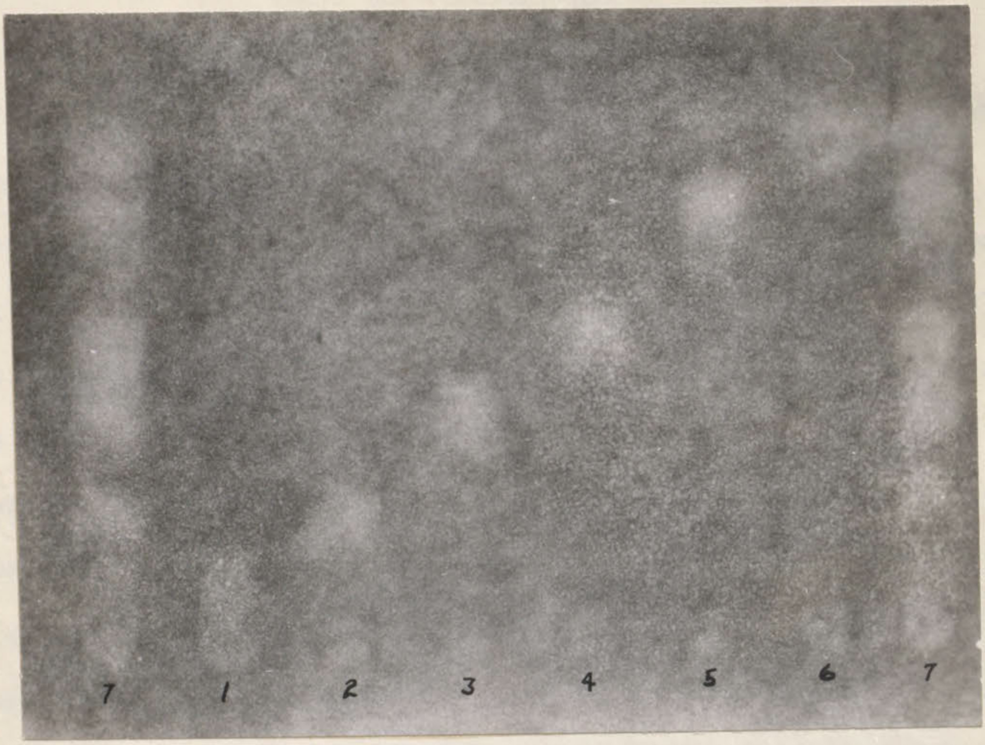

Fig. 3. Filter Paper Chromatogram.

Legend:

1. Oxalic acid.
2. Tartaric acid.
3. Citric acid.
4. Malic acid.
5. Malonic acid.
6. Succinic acid.
7. Mixture of the six acids listed above.

concentration in the original solution is within a range of 10 ug./0.01 ml. to 50 ug./0.02 ml. Succinic and malonic acids give good spots at 60 ug./0.01 ml. while the other acids are diffused at concentrations above 50 ug./ 0.02 ml. The $R_f$ values of the acids increase slightly with increase in acid concentration.

The effect of varying the concentration of formic acid in the solvent also was studied. The data obtained are presented in Table X. Examination of the results shows that good spots for all the six acids were obtained from concentrations of 15 ug./0.01 ml. to 30 ug./0.01 ml. when 3.0 to 3.5 moles of formic acid per liter of solvent were present. The spots produced by acids of concentrations of 15 ug./0.01 ml. to 60 ug./0.01 ml. are slightly better when 4.0 moles of formic acid per liter of solvent are present. At oxalic acid concentrations greater than 30 ug./0.01 ml. the comet produced tends to be less tailed in solvent containing 4.0 moles of formic acid per liter than it is when a lower formic acid concentration is used. The $R_f$ values of the acids decrease slightly as the concentration of formic acid is increased in the solvent.

Other solvents were substituted for n-butanol in an attempt to determine whether or not "two dimensional" filter paper chromatography would be feasible. Table XI records the data of these trials. The response of the acids to these solvents differs from their response to n-butanol only in the distance they move up the paper.

## TABLE IX

## $R_f$ VALUES OF ORGANIC ACIDS WITH VARIOUS CONCENTRATIONS AND VOLUMES APPLIED

| Acid | Concentration | $R_f$ Value | Remarks |
|---|---|---|---|
| Succinic | 10 ug./0.02 ml. | 0.71-0.78 | Good spots |
| " | 15 ug./0.01 ml. | 0.72-0.76 | " " |
| " | 20 ug./0.01 ml. | 0.72-0.73 | " " |
| " | 25 ug./0.01 ml. | 0.71-0.73 | " " |
| " | 30 ug./0.01 ml. | 0.74-0.78 | " " |
| " | 30 ug./0.02 ml. | 0.72-0.75 | " " |
| " | 40 ug./0.02 ml. | 0.71-0.81 | " " |
| " | 50 ug./0.02 ml. | 0.76-0.83 | " " |
| " | 60 ug./0.01 ml. | 0.80 | " " |
| " | 60 ug./0.02 ml. | 0.76-0.81 | " " |
| " | 120 ug./0.02 ml. | 0.79-0.84 | Diffused with malonic acid spot |
| Malonic | 10 ug./0.02 ml. | 0.56-0.66 | Good spots |
| " | 15 ug./0.01 ml. | 0.61-0.62 | " " |
| " | 20 ug./0.01 ml. | 0.60-0.61 | " " |
| " | 20 ug./0.02 ml. | 0.55-0.65 | " " |
| " | 25 ug./0.01 ml. | 0.56-0.61 | " " |
| " | 30 ug./0.01 ml. | 0.62-0.65 | " " |
| " | 30 ug./0.02 ml. | 0.57-0.63 | " " |
| " | 40 ug./0.02 ml. | 0.59-0.70 | " " |
| " | 50 ug./0.02 ml. | 0.71-0.76 | " " |
| " | 60 ug./0.01 ml. | 0.68 | " " |
| " | 60 ug./0.02 ml. | 0.65-0.67 | " " |
| " | 120 ug./0.02 ml. | 0.68-0.72 | Diffused with succinic acid spot |

## TABLE IX CONTINUED

| Acid | Concentration | $R_f$ Value | Remarks |
|---|---|---|---|
| Malic | 10 ug./0.02 ml. | 0.41-0.52 | Good spots |
| " | 15 ug./0.01 ml. | 0.44 | " " |
| " | 20 ug./0.01 ml. | 0.44 | " " |
| " | 20 ug./0.02 ml. | 0.43-0.53 | " " |
| " | 25 ug./0.01 ml. | 0.42-0.44 | " " |
| " | 30 ug./0.01 ml. | 0.44-0.46 | " " |
| " | 30 ug./0.02 ml. | 0.43-0.49 | " " |
| " | 40 ug./0.02 ml. | 0.43-0.50 | " " |
| " | 50 ug./0.02 ml. | 0.49-0.55 | " " |
| " | 60 ug./0.01 ml. | 0.46 | Diffused with citric acid spot |
| " | 60 ug./0.02 ml. | 0.48 | Diffused with citric acid spot |
| " | 120 ug./0.02 ml. | 0.50 | Diffused with citric acid spot |
| Citric | 10 ug./0.02 ml. | 0.30-0.42 | Good spots |
| " | 15 ug./0.01 ml. | 0.33-0.34 | " " |
| " | 20 ug./0.01 ml. | 0.34-0.35 | " " |
| " | 20 ug./0.02 ml. | 0.32-0.42 | " " |
| " | 25 ug./0.01 ml. | 0.31-0.36 | " " |
| " | 30 ug./0.01 ml. | 0.33-0.37 | " " |
| " | 30 ug./0.02 ml. | 0.34-0.36 | " " |
| " | 40 ug./0.02 ml. | 0.34-0.39 | " " |
| " | 50 ug./0.01 ml. | 0.34-0.39 | " " |
| " | 60 ug./0.01 ml. | 0.39 | Diffused with malic acid spot |
| " | 60 ug./0.02 ml. | 0.38-0.40 | Diffused with malic acid spot |
| " | 120 ug./0.02 ml. | 0.44-0.45 | Diffused with malic and tartaric acid spots |

## TABLE IX CONTINUED

| Acid | Concentration | $R_f$ Value | Remarks |
|---|---|---|---|
| Tartaric | 10 ug./0.02 ml. | 0.17-0.28 | Good spots |
| " | 15 ug./0.01 ml. | 0.20-0.26 | " " |
| " | 20 ug./0.01 ml. | 0.22-0.26 | " " |
| " | 20 ug./0.02 ml. | 0.19-0.29 | " " |
| " | 25 ug./0.01 ml. | 0.19-0.26 | " " |
| " | 30 ug./0.01 ml. | 0.21-0.24 | " " |
| " | 30 ug./0.02 ml. | 0.24-0.26 | " " |
| " | 40 ug./0.02 ml. | 0.22-0.24 | " " |
| " | 50 ug./0.02 ml. | 0.22-0.25 | " " |
| " | 60 ug./0.01 ml. | 0.26 | Diffused with citric and oxalic acid spots |
| " | 60 ug./0.02 ml. | 0.27-0.28 | Diffused with citric and oxalic acid spots |
| " | 120 ug./0.02 ml. | 0.25-0.29 | Diffused with citric and oxalic acid spots |
| Oxalic | 10 ug./0.02 ml. | 0.06 | Well defined comet |
| " | 15 ug./0.01 ml. | 0.04-0.05 | " " " |
| " | 20 ug./0.01 ml. | 0.05-0.06 | " " " |
| " | 20 ug./0.02 ml. | 0.05 | " " " |
| " | 25 ug./0.01 ml. | 0.05 | " " " |
| " | 30 ug./0.01 ml. | 0.06-0.07 | " " " |
| " | 30 ug./0.02 ml. | 0.06-0.07 | " " " |
| " | 40 ug./0.02 ml. | 0.06-0.07 | Fairly well defined comet |
| " | 50 ug./0.02 ml. | 0.06 | Fairly well defined comet |
| " | 60 ug./0.01 ml. | 0.12 | Diffused with tartaric acid spot |
| " | 60 ug./0.02 ml. | 0.10-0.13 | Diffused with tartaric acid spot |
| " | 120 ug./0.02 ml. | 0.14 | Diffused with tartaric acid spot |

## TABLE X

## $R_f$ VALUE OF ACIDS WITH VARYING CONCENTRATIONS OF FORMIC ACID IN SOLVENT

| Acid | Moles of HCOOH in n-butanol/ liter | $R_f$ Value | Remarks |
|---|---|---|---|
| Succinic | 3.0 | 0.78-0.83 | Good spots up to 50 ug./0.01 ml. Spot for 60 ug./0.01 ml. rather diffuse. |
| " | 3.5 | 0.75-0.85 | Good spots at all concentrations of acids. Better definition of spots than with 3.0 moles of HCOOH in the solvent. A slight diffusion of spot at 60 ug./0.01 ml. with the malonic acid spot. |
| " | 4.0 | 0.76-0.80 | Good spots at all concentrations of acids. Spots very slightly better defined than those produced when 3.5 moles HCOOH used. Slight diffusion of spot at 60 ug./0.01 ml. with malonic acid spot. |
| Malonic | 3.0 | 0.69-0.75 | Good spots to 30 ug./0.01 ml. Spots of 50 and 60 ug./0.01 ml. poor and diffused. |
| " | 3.5 | 0.65-0.75 | Good spots to 30 ug./0.01 ml. Spots of 50 and 60 ug./0.01 ml. slightly diffused with succinic. By and large, the spots are better than when 3.0 moles HCOOH were used. |
| " | 4.0 | 0.67-0.68 | Same as with 3.5 moles HCOOH in the solvent. |
| Malic | 3.0 | 0.54-0.59 | Good spots to 30 ug./0.01 ml. Spots of 50 and 60 ug./0.01 ml. diffused with citric acid spot. |
| " | 3.5 | 0.48-0.58 | Same as with 3.0 moles HCOOH in the solvent. |

TABLE X CONTINUED

| Acid | Moles of HCOOH in n-butanol/ liter | $R_f$ Value | Remarks |
|---|---|---|---|
| Malic | 4.0 | 0.49-0.50 | Good spots to 50 ug./0.01 ml. Spot of 60 ug./0.01 ml. slightly diffused with citric acid spot. |
| Citric | 3.0 | 0.47-0.52 | Good spots to 30 ug./0.01 ml. Spots of 50 and 60 ug./0.01 ml. diffused with malic acid spot. |
| " | 3.5 | 0.37-0.48 | Same as with 3.0 moles HCOOH in the solvent. |
| " | 4.0 | 0.37-0.41 | Good spots to 50 ug./0.01 ml. Spots of 60 ug./0.01 ml. was very slightly diffused with malic and tartaric acid spots. |
| Tartaric | 3.0 | 0.34-0.36 | Good spots to 50 ug./0.01 ml. |
| " | 3.5 | 0.26-0.29 | Good spots to 60 ug./0.01 ml. |
| " | 4.0 | 0.24-0.29 | Good spots to 60 ug./0.01 ml. |
| Oxalic | 3.0 | 0.09-0.13 | Good comets to 30 ug./0.01 ml. Other comets were diffused with tartaric. |
| " | 3.5 | 0.07-0.10 | Same as with 3.0 moles HCOOH in the solvent. |
| " | 4.0 | 0.08-0.10 | Good comets to 50 ug/0.01 ml. Comet of 60 ug./0.01 ml. had diffused into the tartaric acid spot. |

All acids take the same relative positions on the paper and thus a "two dimensional" run employing any two of the solvents studied would not be of any particular advantage. In addition, the results are not as good as those obtained when n-butanol is used, and therefore these other solvents were not used further. In addition to the solvents which are listed in Table XI, the following were tried but very poor results were obtained: butyl acetate, butyl propionate, toluene, chloroform, p-dioxane, and octyl alcohol.

The $R_f$ values of other acids are recorded in Table XII. It is worthy of note that maleic (cis) acid and fumaric (trans) acid are completely separated by this method.

A solution containing 10 mg. each of succinic, malonic, oxalic, malic, citric, and tartaric acids was separated by silica gel partition chromatography, during which successive 50 ml. portions of the eluate were collected. These fractions, after titrating with a 0.01 N barium hydroxide solution to pH 8.4 were placed in separate separatory funnels and the aqueous phases were collected and evaporated to dryness. The calculated amount of 2 N sulphuric acid was added to each residue to liberate the acid from the barium salt. A few millilitres of distilled water were added to bring the acids into solution and the solution and barium sulphate precipitate

## TABLE XI

## $R_f$ VALUES OF ACIDS USING VARIOUS SOLVENTS

| Solvent | Acid | $R_f$ Value | Remarks |
|---|---|---|---|
| Propyl alcohol | Succinic | 0.70 | Good spot |
| " " | Malonic | 0.62 | " " |
| " " | Malic | 0.47 | " " |
| " " | Citric | 0.34 | " " |
| " " | Tartaric | 0.19 | " " |
| " " | Oxalic | 0.11 | Good comet |
| 100 per cent ethanol | Succinic | 0.84 | Good spot |
| " " | Malonic | 0.78 | " " |
| " " | Malic | 0.64 | " " |
| " " | Citric | - | No spot, tailed up the paper |
| " " | Tartaric | 0.39 | Good spot |
| " " | Oxalic | 0.16 | Good comet |
| 95 per cent ethanol | Succinic | 0.86 | Good spot |
| " " | Malonic | 0.82 | " " |
| " " | Malic | - | Diffusion of acid over a large area |
| " " | Citric | - | No spot, tailed up the paper |
| " " | Tartaric | 0.44 | Good spot |
| " " | Oxalic | 0.29 | Good comet |
| Methyl-amyl alcohol | Succinic | 0.46 | Good spot |
| " " | Malonic | 0.38 | " " |
| " " | Malic | 0.19 | " " |
| " " | Citric | 0.12 | " " |
| " " | Tartaric | 0.12 | Fair spot with slight tailing |
| " " | Oxalic | 0.09 | Good comet |

## TABLE XII

## $R_f$ VALUES OF OTHER ACIDS

| Acid | Concentration/0.01 ml. | $R_f$ Value | Remarks |
|---|---|---|---|
| Lactic | 30 ug. | 0.64-0.76 | Good spots |
| " | 60 ug. | 0.76 | Good spot |
| Glycollic | 30 ug. | 0.49-0.54 | Good spots |
| " | 60 ug. | 0.55 | Good spot |
| Hydracrylic | 30 ug. | 0.80-0.82 | Good spots |
| " | 60 ug. | 0.80 | Good spot |
| Gallic | 30 ug. | 0.57-0.59 | Good spots |
| Cinnamic | 30 ug. | 0.93-0.94 | Fair spots |
| Phthalic | 30 ug. | 0.84-0.86 | Good spots |
| Fumaric | 30 ug. | 0.82-0.86 | Good spots |
| Maleic | 30 ug. | 0.46-0.49 | Good spots |
| Hippuric | 30 ug. | 0.85-0.88 | Good spots |
| Benzoic | 30 ug. | 0.93-0.94 | Poor spots |

were transferred to small vials. An aliquot (0.02 ml.) of each solution was placed on a filter paper cylinder to identify the acids present in the various fractions.

The data from this experiment are contained in Table XIII. The percentage recovery is satisfactory and the six acids are readily identified by their $R_f$ values when a known solution of the acids is run as a standard on the same paper. This standard solution is necessary because if a known solution of acids is placed on two separate filter papers and these are run at the same time, the $R_f$ values of the same acid on the different papers may be different. This is borne out in Table IX where it is seen that the range of $R_f$ values of one acid may overlap with the $R_f$ values of the acid immediately ahead of or behind it. This is especially true with fractions from the silica column in which the concentration of the acid is unknown. A fraction containing a large amount of citric acid if spotted on a paper chromatogram often has an $R_f$ value similar to a small concentration of malic acid. If these two fractions were spotted on different paper chromatograms their individual $R_f$ values, in the absence of a standard solution, would not be conclusive evidence for the identification of the acid in the fraction. However, when a known solution of acids is spotted beside an unknown fraction, identification of the unknown acid readily can be made.

Succinic and malonic acids are not completely separated by the silica gel partition chromatogram, but by the latter method when 50 ml. portions of the eluate were collected a fraction containing only one of these acids has been obtained. Oxalic acid leaves the silica column after malonic acid, but on the filter paper chromatogram it appears as a comet shaped spot with a low $R_f$ value.

The combination of these two partition chromatogram techniques has been used successfully on the leaf leachates for the isolation and identification of organic acids.

## TABLE XIII

### SEPARATION AND IDENTIFICATION OF ORGANIC ACIDS BY COMBINATION PROCEDURE

| Fraction | % n-butanol Solvent | Ml. of Barium Hydroxide | Rf Value | Acid | % Recovery |
|---|---|---|---|---|---|
| 1 | 5 | 3.60 | 0.74 | Succinic | 103.3 |
| 2 | 5 | 3.75 | 0.74 | Succinic | |
| 3 | 5 | 4.30 | 0.74 | Succinic | |
| 4 | 10 | 15.70 | 0.74-0.61 | Succinic<br>Malonic | |
| 5 | 10 | 10.00 | 0.61 | Malonic | |
| 6 | 10 | 6.40 | Comet shape | Oxalic | 98.6 |
| 7 | 20 | 9.25 | Comet shape | Oxalic | |
| 8 | 20 | 1.25 | - | - | |
| 9 | 20 | 4.75 | 0.45 | Malic | 94.5 |
| 10 | 20 | 5.25 | 0.45 | Malic | |
| 11 | 35 | 4.10 | 0.45 | Malic | |
| 12 | 35 | 9.00 | 0.33 | Citric | 91.1 |
| 13 | 35 | 4.00 | 0.33 | Citric | |
| 14 | 35 | 1.00 | - | - | |
| 15 | 35 | 5.30 | 0.20 | Tartaric | 83.1 |
| 16 | 35 | 2.15 | 0.20 | Tartaric | |
| 17 | 35 | 3.62 | 0.20 | Tartaric | |

The determination of partition coefficients:- Acids move at different rates through a partition chromatogram because each acid has a different partition coefficient between the solvents employed, and if this difference is large enough the acids are completely separated. In the silica gel partition chromatogram the six acids moved from the column in the following order: succinic, malonic, oxalic, malic, citric, and finally tartaric acid. When the same acids were separated on filter paper, oxalic acid appeared as a comet shaped spot, moving much slower than tartaric acid. It was decided to determine the partition coefficient of these acids between the solvents used in each method to see if the presence of formic acid in the n-butanol greatly altered the partition coefficient of oxalic acid, and also to correlate the movement of the other acids in both methods with their partition coefficients.

The partition coefficients of the acids between n-butanol and water were determined in the following manner: A 0.1 N solution of each acid was prepared and a 10 ml. aliquot of this solution was pipetted into a clean separatory funnel, to which was added by means of a pipette, a 10 ml. aliquot of n-butanol. The separatory funnel was securely stoppered and shaken. The water and butanol were allowed to separate into two layers, and each layer was drawn off into separate dry, 125 ml. Erlenmeyer flasks. These solutions were titrated with 0.0970 N sodium hydroxide to a pink end point after 2 drops of 0.5 per cent phenolphthalein solution had been added. A little absolute ethanol was added to each butanol titration to make the solution monophasic. The partition coefficients can be calculated from this data.

The partition coefficients of the acids between n-butanol, containing 3.0 moles of formic acid per liter, and water were also determined. The procedure was the same as above except that the two layers, when collected in separate Erlenmeyer flasks, were evaporated to dryness under reduced pressure at room temperature to remove the formic acid. About 25 ml. of distilled water and 2 drops of phenolphthalein indicator were added to each flask, and the solution was titrated to a faint pink end-point with 0.0970 N sodium hydroxide.

In Table XIV are recorded the data for determining the partition coefficients between n-butanol and water. It is seen that the partition coefficients increase progressively from succinic to tartaric acid, and as would be expected, the values of the partition coefficients vary inversely with the rate of movement of the acids in the silica gel partition column.

The partition coefficients of the acids between n-butanol containing 3.0 moles of formic acid per liter, and water (Table XV) agree fairly well with the movement of the acids on filter paper. The presence of formic acid increases the amount of acid passing into the water phase, and therefore the partition coefficients of all the acids are increased. In the case of oxalic acid however, the formic acid has a much greater effect on its partition coefficient, the value of which is just less than for citric acid. From these partition coefficients oxalic acid would be expected to move on filter paper just ahead of citric acid, which however is not the case. It appears that the partition coefficient is not the only prominent factor in the movement of an acid on filter paper, and probably adsorption of the acid on the paper plays an important part. It might also be pointed out that the small amount of water present in the solvent system of filter paper chromatography is likely not duplicated closely enough when partition coefficients are determined on equal amounts of the two solvents (water and n-butanol) as has been done in this procedure.

### 4. Other Methods of Identifying Acids in Fractions

Buffer-index curves were used for further confirmation of the identity of the isolated acids using the method as outlined by Small (28).

Solutions of known acids or acidic fractions from the chromatograms were titrated with small aliquots of 0.01 N KOH and the changes in pH were noted.

The buffer-index is a definite direct measure of the buffer capacity and is calculated as dB/dpH, where dB is the number of gram-equivalents of acid or base required per liter for the observed change of pH per liter of buffer solution. The buffer-index is calculated from the following formula:

$$\text{Buffer-index} = \frac{\text{Normality of base x ml. of base}}{\text{ml. of buffer solution x change in pH}}$$

The buffer index curves are characteristic for each acid in respect to the general shape and the pH at which maximum buffering occurs.

Spot tests as outlined by Feigl (29) were applied to the acid fractions after rendering them as colorless as possible by treatment with Norit-A.

## TABLE XIV

## THE DETERMINATION OF PARTITION COEFFICIENTS OF ORGANIC ACIDS BETWEEN BUTANOL AND DISTILLED WATER

| Acid | Grams acid/ 10 ml. $H_2O$ | Volume of 0.0979 N NaOH required in |  | Grams acid calculated to be in |  | Per cent Acid accounted for | Partition Coefficient |
|---|---|---|---|---|---|---|---|
|  |  | Aqueous phase | Butanol phase | Aqueous phase | Butanol phase |  |  |
| Succinic | 0.0590 g. | 4.5 ml. | 5.85 ml. | 0.0257 g. | 0.0330 g. | 99.4 | 0.77 |
| " | 0.0590 g. | 4.5 ml. | 5.84 ml. | 0.0257 g. | 0.0330 g. | 99.4 | 0.77 |
| Malonic | 0.0520 g. | 5.15 ml. | 4.90 ml. | 0.0259 g. | 0.0244 g. | 96.7 | 1.06 |
| " | 0.0520 g. | 5.30 ml. | 4.85 ml. | 0.0267 g. | 0.0244 g. | 98.2 | 1.09 |
| Oxalic | 0.0630 g. | 6.65 ml. | 3.40 ml. | 0.0406 g. | 0.0207 g. | 97.3 | 1.96 |
| " | 0.0630 g. | 6.65 ml. | 3.30 ml. | 0.0406 g. | 0.0207 g. | 97.3 | 1.96 |
| Malic | 0.0670 g. | 6.90 ml. | 3.30 ml. | 0.0448 g. | 0.0214 g. | 98.8 | 2.08 |
| " | 0.0670 g. | 6.90 ml. | 3.30 ml. | 0.0448 g. | 0.0214 g. | 98.8 | 2.08 |
| Citric | 0.0640 g. | 6.95 ml. | 2.95 ml. | 0.0428 g. | 0.0183 g. | 95.4 | 2.33 |
| " | 0.0640 g. | 6.95 ml. | 3.00 ml. | 0.0438 g. | 0.0185 g. | 95.7 | 2.31 |
| Tartaric | 0.0910 g. | 7.75 ml. | 2.34 ml. | 0.0684 g. | 0.0206 g. | 97.8 | 3.32 |
| Fumaric | 0.0580 g. | 1.70 ml. | 8.35 ml. | 0.0093 g. | 0.0469 g. | 96.8 | 0.19 |
| Maleic | 0.0580 g. | 5.20 ml. | 5.15 ml. | 0.0292 g. | 0.0284 g. | 99.3 | 1.02 |
| Glycollic | 0.0761 g. | 7.10 ml. | 3.20 ml. | 0.0525 g. | 0.0236 g. | 100.0 | 2.22 |

# TABLE XV

## THE DETERMINATION OF PARTITION COEFFICIENTS OF ORGANIC ACIDS BETWEEN BUTANOL CONTAINING 3.0 MOLES OF HCOOH/LITER AND DISTILLED WATER

| Acid | Grams acid/ 10 ml. $H_2O$ | Volume of 0.0970 N NaOH required in | | Grams acid calculated to be in | | Per cent Acid accounted for | Partition Coefficient |
|---|---|---|---|---|---|---|---|
| | | Aqueous phase | Butanol phase | Aqueous phase | Butanol phase | | |
| Succinic | 0.0590 g. | 6.10 ml. | 4.20 ml. | 0.0348 g. | 0.0241 g. | 99.8 | 1.44 |
| " | 0.0590 g. | 6.20 ml. | 4.12 ml. | 0.0354 g. | 0.0235 g. | 99.8 | 1.51 |
| Malonic | 0.0520 g. | 6.95 ml. | 3.30 ml. | 0.0348 g. | 0.0166 g. | 98.8 | 2.09 |
| " | 0.0520 g. | 6.87 ml. | 3.50 ml. | 0.0346 g. | 0.0171 g. | 99.4 | 2.04 |
| Malic | 0.0670 g. | 8.05 ml. | 2.25 ml. | 0.0523 g. | 0.0146 g. | 99.8 | 3.58 |
| " | 0.0670 g. | 8.00 ml. | 2.28 ml. | 0.0519 g. | 0.0147 g. | 99.4 | 3.53 |
| Citric | 0.0640 g. | 7.85 ml. | 2.00 ml. | 0.0487 g. | 0.0124 g. | 95.4 | 3.92 |
| " | 0.0640 g. | 7.90 ml. | 1.95 ml. | 0.0490 g. | 0.0122 g. | 95.6 | 4.01 |
| Oxalic | 0.0630 g. | 8.05 ml. | 2.20 ml. | 0.0492 g. | 0.0134 g. | 99.3 | 3.67 |
| " | 0.0630 g. | 7.80 ml. | 2.10 ml. | 0.0479 g. | 0.0126 g. | 96.1 | 3.80 |
| Fumaric | 0.0580 g. | 2.90 ml. | 7.45 ml. | 0.0162 g. | 0.0418 g. | 100.0 | 0.38 |
| " | 0.0580 g. | 2.80 ml. | 7.22 ml. | 0.0052 g. | 0.0406 g. | 79.0 | 0.12 |
| Maleic | 0.0580 g. | 6.52 ml. | 3.63 ml. | 0.0366 g. | 0.0203 g. | 98.1 | 1.80 |
| " | 0.0580 g. | 6.52 ml. | 3.52 ml. | 0.0366 g. | 0.0197 g. | 99.0 | 1.80 |
| Glycollic | 0.0761 g. | 7.45 ml. | 1.45 ml. | 0.0548 g. | 0.0107 g. | 86.1 | 5.12 |
| " | 0.0761 g. | 7.72 ml. | 1.40 ml. | 0.0570 g. | 0.0107 g. | 89.0 | 5.32 |

# IV EXPERIMENTAL RESULTS AND DISCUSSION

## 1. Preliminary Study of The Leachates

The volumes of the leachates, after filtering to remove any solid materials present, were measured. The data obtained from the determinations that were made on aliquot samples are recorded in Tables XVI, XVII, XVIII, and XIX.

While a detailed study of these results has not been carried out, it is obvious that the leachates of the four species became progressively more alkaline during the eleven month period from October to the following September. This increase in pH was in the range of 1.5 to 2.0 pH units. From the last of April onward, the birch leachates were predominantly alkaline. The poplar leachate varied considerably but maintained a pH of about 7.0 from July to the end of the collection period. Beech and maple leachates were consistently acid in reaction. This trend toward decreased acidity also was shown in the titratable acidities or alkalinities per 100 ml. of leachate, although these values were highly variable. Beech, maple and poplar showed a more pronounced change than birch. The total titratable acidities (to pH 7.0) of beech, maple, and poplar leachates collected during the whole leaching period, when calculated in terms of calcium carbonate equivalents, were found to be equivalent to 643, 4248, and 594 pounds of calcium carbonate per acre respectively.

## TABLE XVI

## PROPERTIES OF BEECH LEACHATES

| Date Collected | Volume (l.) | pH | Titratable Acidity or Alkalinity as ml. of 0.1 N NaOH or HCl per 100 ml. | Total | Grams Ash/ 100 ml. | Total Ash |
|---|---|---|---|---|---|---|
| Oct. 29 | 3.69 | 5.58 | 1.16 | 42.80 | 0.1162 | 4.2888 |
| " 30 | 2.33 | 5.81 | 0.44 | 10.23 | 0.0185 | 0.4311 |
| Nov. 8 | 21.00 | 5.58 | 0.61 | 128.10 | 0.0053 | 0.1113 |
| " 9 | 19.16 | 5.80 | 0.09 | 17.24 | 0.0030 | 0.5748 |
| " 11-12 | 22.53 | 5.50 | 0.18 | 40.55 | 0.0069 | 1.5546 |
| Apr. 8 | 1.40 | 5.72 | 0.45 | 6.31 | 0.0130 | 0.1680 |
| " 12 | 5.33 | 5.53 | 0.32 | 17.00 | 0.0093 | 0.4957 |
| " 15 | 3.56 | 6.48 | 0.52 | 18.33 | 0.0111 | 0.3952 |
| " 17 | 3.18 | 6.08 | 0.22 | 7.03 | 0.0087 | 0.2767 |
| " 20-21 | 21.30 | 6.21 | 0.17 | 36.21 | 0.0038 | 0.8094 |
| " 30 | 2.73 | 6.20 | 0.25 | 6.69 | 0.0098 | 0.2675 |
| May 8 | 14.52 | 6.22 | 0.12 | 17.13 | 0.0026 | 0.3775 |
| " 10 | 2.65 | 6.27 | 0.11 | 2.86 | 0.0061 | 0.1617 |
| " 15 | 4.03 | 6.30 | 0.22 | 8.71 | 0.0111 | 0.4473 |
| " 18 | 4.35 | 6.20 | 0.12 | 5.13 | 0.0076 | 0.3306 |
| " 25 | 3.90 | 6.20 | 0.07 | 2.89 | 0.0098 | 0.3822 |
| June 9-10 | 17.36 | 5.86 | 0.05 | 8.33 | 0.0061 | 1.0590 |
| " 21 | 3.67 | 6.48 | 0.20 | 7.49 | 0.0220 | 0.8074 |
| " 23-25 | 9.34 | 6.30 | 0.31 | 29.33 | 0.0161 | 1.5037 |
| " 28 | 13.50 | 6.10 | 0.19 | 25.69 | 0.0120 | 1.6224 |
| July 5 | 3.88 | 6.60 | 0.17 | 6.52 | 0.0271 | 1.0515 |
| " 6 | 16.00 | 6.35 | 0.09 | 14.34 | 0.0112 | 1.7920 |
| " 12 | 16.00 | 6.40 | 0.12 | 18.79 | 0.0054 | 0.8748 |
| " 18-21 | 5.88 | 6.60 | 0.20 | 11.88 | 0.0125 | 0.7350 |
| " 23 | 15.40 | 7.20 | 0.02 | 3.39 | 0.0024 | 0.3960 |
| " 26 | 7.82 | 6.30 | 0.09 | 7.04 | 0.0074 | 0.5787 |
| " 31 | 4.00 | 6.28 | 0.11 | 4.52 | 0.0161 | 0.6400 |
| Aug. 3 | 4.76 | 6.32 | 0.10 | 4.95 | 0.0129 | 0.6140 |
| " 6 | 8.49 | 6.48 | 0.11 | 9.51 | 0.0182 | 1.5452 |
| " 12-14 | 24.20 | 6.48 | 0.05 | 11.13 | 0.0052 | 1.2584 |
| " 30 | 6.87 | 6.60 | 0.13 | 8.73 | 0.0206 | 1.4152 |
| Sept. 10 | 2.66 | 6.60 | 0.11 | 2.98 | 0.0269 | 0.7155 |

## TABLE XVII

## PROPERTIES OF BIRCH LEACHATES

| Date Collected | Volume (l.) | pH | Titratable Acidity or Alkalinity as ml. of 0.1 N NaOH or HCl per 100 ml. | Total | Grams Ash/ 100 ml. | Total Ash |
|---|---|---|---|---|---|---|
| Oct. 29 | 4.18 | 5.65 | 3.20 | 133.17 | 0.1636 | 6.8303 |
| " 30 | 0.87 | 6.00 | 1.04 | 9.04 | 0.0629 | 0.5472 |
| Nov. 8 | 22.09 | 6.04 | 0.85 | 187.77 | 0.0122 | 2.6949 |
| " 9 | 18.38 | 6.20 | 0.13 | 22.98 | 0.0076 | 1.3969 |
| " 11-12 | 22.30 | 6.03 | 0.61 | 136.03 | 0.0140 | 3.1220 |
| April 8 | 1.59 | 7.00 | - | - | 0.0227 | 0.3609 |
| " 12 | 5.88 | 6.80 | 0.05 | 2.88 | 0.0180 | 1.1113 |
| " 15 | 2.86 | 7.27 | 0.30 | 8.67 | 0.0315 | 0.9009 |
| " 17 | 3.06 | 6.98 | 0.36 | 11.11 | 0.0260 | 0.7956 |
| " 20-21 | 20.50 | 6.98 | 0.05 | 11.07 | 0.0119 | 2.4395 |
| " 30 | 3.05 | 7.40 | 0.36 | 11.07 | 0.0316 | 0.9638 |
| May 8 | 16.00 | 7.00 | - | - | 0.0133 | 2.1280 |
| " 10 | 2.75 | 7.00 | - | - | 0.0331 | 0.9103 |
| " 15 | 3.77 | 7.32 | 0.50 | 18.70 | 0.0490 | 1.8473 |
| " 18 | 3.27 | 7.25 | 0.63 | 20.54 | 0.0471 | 1.5378 |
| " 25 | 4.89 | 7.50 | 0.81 | 39.66 | 0.0635 | 3.1052 |
| June 9-10 | 20.16 | 7.25 | 0.25 | 51.21 | 0.0247 | 4.9795 |
| " 21 | 6.25 | 7.38 | 0.54 | 33.75 | 0.0551 | 3.4438 |
| " 23-25 | 10.96 | 7.40 | 1.10 | 120.78 | 0.0528 | 5.2589 |
| " 28 | 14.94 | 7.20 | 0.32 | 48.41 | 0.0437 | 6.5288 |
| July 5 | 6.35 | 7.25 | 0.52 | 32.89 | 0.0504 | 3.2004 |
| " 6 | 8.32 | 7.12 | 0.44 | 81.16 | 0.0269 | 4.9281 |
| " 12 | 12.00 | 7.00 | - | - | 0.0253 | 3.0360 |
| " 18-21 | 7.35 | 7.41 | 0.54 | 39.69 | 0.0373 | 2.7416 |
| " 23 | 16.18 | 7.10 | 0.03 | 5.18 | 0.0184 | 2.9771 |
| " 26 | 12.94 | 7.50 | 0.21 | 27.17 | 0.0237 | 3.0668 |
| " 31 | 6.72 | 7.43 | 0.32 | 21.77 | 0.0288 | 1.9354 |
| Aug. 3 | 6.26 | 7.36 | 0.29 | 18.28 | 0.0271 | 1.7591 |
| " 6 | 7.73 | 7.32 | 0.38 | 28.94 | 0.0271 | 2.0948 |
| " 12-14 | 29.21 | 7.28 | 0.22 | 63.09 | 0.0252 | 7.3609 |
| " 30 | 9.06 | 7.40 | 0.50 | 45.03 | 0.0285 | 2.5821 |
| Sept. 10 | 2.78 | 7.75 | 0.78 | 21.68 | 0.0398 | 1.1604 |

## TABLE XVIII

## PROPERTIES OF MAPLE LEACHATES

| Date Collected | Volume (l.) | pH | Titratable Acidity as ml. of 0.1 N NaOH | | Grams Ash/ 100 ml. | Total Ash |
|---|---|---|---|---|---|---|
| | | | per 100 ml. | Total | | |
| Oct. 29 | 3.70 | 4.70 | 3.55 | 131.17 | 0.0493 | 1.8241 |
| " 30 | 1.20 | 4.70 | 3.31 | 39.66 | 0.0521 | 0.6252 |
| Nov. 8 | 20.54 | 4.75 | 1.29 | 264.97 | 0.0133 | 2.7318 |
| " 9 | 22.47 | 4.50 | 0.50 | 112.35 | 0.0072 | 1.6208 |
| " 11-12 | 22.88 | 4.02 | 1.74 | 398.11 | 0.0149 | 3.4091 |
| April 8 | 1.77 | 4.68 | 2.16 | 38.16 | 0.0247 | 0.4372 |
| " 12 | 6.55 | 4.50 | 1.72 | 112.33 | 0.0179 | 1.1725 |
| " 15 | 3.10 | 4.69 | 1.81 | 56.20 | 0.0197 | 0.6107 |
| " 17 | 3.26 | 4.39 | 1.28 | 41.86 | 0.0164 | 0.5346 |
| " 20-21 | 20.25 | 4.96 | 0.74 | 148.84 | 0.0076 | 1.5390 |
| " 30 | 4.88 | 5.02 | 0.92 | 44.95 | 0.0146 | 0.7125 |
| May 8 | 16.25 | 5.02 | 0.68 | 109.85 | 0.0050 | 0.8125 |
| " 10 | 3.62 | 5.35 | 0.71 | 25.56 | 0.0099 | 0.3584 |
| " 15 | 5.25 | 5.60 | 0.67 | 34.97 | 0.0140 | 0.7350 |
| " 18 | 5.36 | 5.32 | 0.76 | 40.47 | 0.0117 | 0.6271 |
| " 25 | 4.44 | 5.00 | 1.29 | 57.45 | 0.0133 | 0.5905 |
| June 9-10 | 17.57 | 5.49 | 0.27 | 47.26 | 0.0044 | 0.7731 |
| " 21 | 3.18 | 5.45 | 1.48 | 47.03 | 0.0313 | 0.9953 |
| " 23-25 | 10.85 | 5.00 | 1.01 | 109.37 | 0.0176 | 1.9096 |
| " 28 | 12.50 | 5.20 | 0.90 | 112.00 | 0.0129 | 1.6125 |
| July 5 | 5.55 | 5.15 | 1.10 | 60.94 | 0.0245 | 1.3598 |
| " 6 | 17.25 | 5.55 | 0.43 | 73.49 | 0.0088 | 1.5180 |
| " 12 | 14.00 | 6.20 | 0.41 | 57.96 | 0.0096 | 1.3440 |
| " 18-21 | 4.66 | 5.82 | 0.90 | 41.75 | 0.0222 | 1.0345 |
| " 23 | 16.30 | 5.65 | 0.22 | 35.37 | 0.0056 | 0.9128 |
| " 26 | 6.96 | 5.60 | 0.62 | 43.15 | 0.0125 | 0.8700 |
| " 31 | 5.56 | 5.52 | 0.86 | 47.93 | 0.0217 | 1.2065 |
| Aug. 3 | 4.80 | 5.50 | 0.87 | 41.95 | 0.0215 | 1.0320 |
| " 6 | 5.40 | 5.66 | 0.80 | 42.93 | 0.0325 | 1.7550 |
| " 12-14 | 29.25 | 5.54 | 0.51 | 14.92 | 0.0117 | 3.4223 |
| " 30 | 7.10 | 6.15 | 0.55 | 39.12 | 0.0310 | 0.2201 |
| Sept. 10 | 3.36 | 6.55 | 0.35 | 11.76 | 0.0323 | 1.0853 |

## TABLE XIX

## PROPERTIES OF POPLAR LEACHATES

| Date Collected | Volume (l.) | pH | Titratable Acidity or Alkalinity as ml. of 0.1 N NaOH or HCl |  | Grams Ash/ 100 ml. | Total Ash |
|---|---|---|---|---|---|---|
|  |  |  | per 100 ml. | Total |  |  |
| Oct. 29 | 4.64 | 5.48 | 4.23 | 196.06 | 0.0732 | 3.3965 |
| " 30 | 0.74 | 5.48 | 1.92 | 14.21 | 0.0770 | 0.5698 |
| Nov. 8 | 22.33 | 5.68 | 0.24 | 62.52 | 0.0165 | 3.6845 |
| " 9 | 20.00 | 5.50 | 0.23 | 45.00 | 0.0086 | 1.7200 |
| " 11-12 | 21.27 | 5.58 | 0.55 | 116.98 | 0.0190 | 4.0413 |
| April 8 | 2.11 | 6.40 | 0.32 | 6.69 | 0.0142 | 0.2996 |
| " 12 | 7.17 | 6.43 | 0.12 | 8.46 | 0.0084 | 0.6023 |
| " 15 | 2.96 | 6.60 | 0.08 | 2.46 | 0.0163 | 0.4825 |
| " 17 | 3.33 | 6.63 | - | - | 0.0112 | 0.3730 |
| " 20-21 | 19.00 | 6.58 | 0.16 | 29.83 | 0.0050 | 0.9500 |
| " 30 | 4.01 | 6.90 | 0.03 | 1.16 | 0.0105 | 0.4211 |
| May 8 | 16.51 | 6.12 | 0.10 | 16.18 | 0.0027 | 0.4458 |
| " 10 | 2.88 | 6.39 | 0.12 | 3.40 | 0.0089 | 0.2563 |
| " 15 | 4.33 | 6.32 | 0.10 | 4.34 | 0.0132 | 0.5848 |
| " 18 | 4.76 | 6.38 | 0.04 | 1.86 | 0.0111 | 0.5284 |
| " 25 | 3.33 | 7.00 | - | - | 0.0147 | 0.4895 |
| June 9-10 | 17.40 | 6.41 | 0.04 | 7.13 | 0.0039 | 0.6786 |
| " 21 | 2.65 | 7.00 | - | - | 0.0253 | 0.6705 |
| " 23-25 | 10.94 | 6.80 | 0.05 | 4.92 | 0.0109 | 1.1925 |
| " 28 | 13.70 | 6.40 | 0.08 | 1.07 | 0.0093 | 1.2741 |
| July 5 | 3.00 | 6.80 | 0.03 | 0.84 | 0.0247 | 0.7410 |
| " 6 | 12.90 | 6.75 | 0.03 | 4.39 | 0.0086 | 1.1094 |
| " 12 | 17.30 | 6.80 | 0.03 | 5.88 | 0.0061 | 1.0553 |
| " 18-21 | 6.14 | 6.89 | 0.12 | 7.12 | 0.0167 | 1.0254 |
| " 23 | 16.40 | 7.00 | - | - | 0.0025 | 0.4100 |
| " 26 | 5.90 | 6.90 | 0.02 | 1.30 | 0.0071 | 0.4189 |
| " 31 | 4.60 | 7.00 | - | - | 0.0166 | 0.6640 |
| Aug. 3 | 3.90 | 6.90 | 0.01 | 0.43 | 0.0148 | 0.5772 |
| " 6 | 6.70 | 7.00 | - | - | 0.0131 | 0.8777 |
| " 12-14 | 28.15 | 6.70 | 0.03 | 8.45 | 0.0054 | 1.5201 |
| " 30 | 6.36 | 7.15 | 0.39 | 25.06 | 0.0177 | 1.1257 |
| Sept. 10 | 2.28 | 7.24 | 0.36 | 8.30 | 0.0237 | 0.5405 |

## TABLE XX

### RESIDUES REMAINING ON THE TRAYS AFTER ELEVEN MONTHS EXPOSURE

| Species | Weight (g.) | Per cent |
|---|---|---|
| Beech | 2790 | 86.7 |
| Birch | 4178 | 69.1 |
| Maple | 2347 | 65.5 |
| Poplar | 2399 | 80.0 |

The birch leachates which had an excess of bases over acids were found to give an equivalent of 96 lbs. of calcium carbonate per acre. The leachates were studied only from October to the following September and, as shown in Table XX, a large amount of residue remained. If these residues had been studied for another year it is probable that all the leachates from them would have become alkaline during the second summer.

The ash content per 100 ml. of leachate during the first rain (October 29th and 30th) is considerably higher for each species than for any subsequent rain. This probably is due, in part at least, to clay particles which adhered to the leaves when they were gathered, and which were washed from the leaves during the first rainfall. During the period of leaching the ash content per 100 ml. of beech leachate increased in general. In the birch leachates the ash content was higher during May and June than at any other time, while the maple leachates were progressively higher in ash content from the last of July onward. The ash content of poplar leachates remained relatively constant throughout the leaching period.

## 2. Concentration of the Leachates

### a. Precipitation Using Lead Acetate

The major part of the leachates of the autumn of 1947 was treated with lead acetate to precipitate the materials in the leachates. The weights of the lead precipitates are given in Table XXI. Several of the leachate collections of the summer of 1948 which were not concentrated by distillation were also treated with lead acetate, but the author did not analyse any of these.

TABLE XXI

LEAD PRECIPITATES FROM LEACHATES 1947

| Species | Weight (grams) |
|---|---|
| Beech | 4.2847 |
| Birch | 12.7820 |
| Maple | 37.1353 |
| Poplar | 17.6280 |

### b. Distillation of the Leachates

In a trial run for the concentration of the acidic material in the leachates, two samples of maple leachate

(October 29th and November 9th, 1947) were combined. The total volume was 3440 ml.

A distillation apparatus, consisting of a 3-liter round bottomed flask, a water cooled condenser and a receiving flask was set up. A few porous plate chips were added and the leachate was distilled by heating over a Meker burner. The distillate was collected in four different flasks and the distillation was stopped when about 25 ml. remained in the distillation flask. This residual material was dark brown in color and a dark precipitate was present. The precipitate was centrifuged off and the titratable acidities were determined on aliquot portions of the supernatant solution and distillates. Table XXII gives the results of this distillation experiment.

## TABLE XXII

## DISTILLATION OF MAPLE LEACHATE

| Solution | | pH | Volume (ml.) | Titratable Acidity as ml. of 0.01 N NaOH | Total Titratable Acidity | Color |
|---|---|---|---|---|---|---|
| Distillate | 1 | 6.30 | 1530 | 0.90/100 ml. | 13.77 | colorless |
| " | 2 | 6.05 | 1050 | 0.93/100 ml. | 9.77 | " |
| " | 3 | 4.90 | 660 | 1.52/100 ml. | 10.03 | " |
| " | 4 | 3.95 | 65 | 1.08/15 ml. | 4.68 | " |
| Supernatant | | 4.02 | 26 | 87.42/5 ml. | 454.58 | dark brown |
| | | | | | 492.83 | |

It is seen that only 7.8 per cent of the total titratable acidity was present in the distillates. Also the distillates become progressively more acidic as the distillation proceeds.

Several of the leachates that were collected during the summer were concentrated by this method. Considerable difficulty was experienced in distilling the birch leachate due to bumping and foaming. The addition of Dow-Corning Anti-Foam 'A' reagent made quite an improvement, but during the latter part of the distillations, all the leachates foamed considerably with the result that some of the concentrated leachate sometimes passed over into the distillate.

The titratable acidities or alkalinities were determined on aliquots of the distillates and the small volume of concentrated leachates remaining in the distillation flasks. It was hoped that the total titratable acidity or alkalinity of the leachate after distillation would not be far different from the total titratable acidity or alkalinity of the leachate when collected from the tray. This however was only found to be so in a few instances as can be seen from Table XXIII.

There are great variations in the acidity of alkalinity accounted for in each species. This is probably due to constitutional changes in the leachate during the long period of heating. Nothing has been done to investigate this possibility. Also the technique of distilling the

# TABLE XXIII

## DISTILLATION OF LEACHATES

| Species | Date of Collection | Leachate | | | Distillate | | | Residue | | | Percentage Titratable Acidity or Alkalinity Accounted for |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | Volume Distilled (ml.) | Total Titratable Acidity or Alkalinity as ml. of 0.01 N | pH | Volume Collected (ml.) | Total Titratable Acidity or Alkalinity as ml. of 0.01 N | pH | Volume (ml.) | Total Titratable Acidity or Alkalinity as ml. of 0.01 N | |
| Beech | 18-5-48 | 6.2 | 2330 | 27.4 (NaOH) | 5.1 | 2150 | 8.4 (NaOH) | 5.6 | 74.5 | 18.1 (NaOH) | 96.7 |
| " | 25-5-48 | 6.2 | 1990 | 14.0 " | 5.9 | 1750 | 15.2 " | 6.0 | 70.0 | 15.4 " | 218.5 |
| " | 9-6-48 | 5.9 | 6000 | 32.3 " | 5.7 | 5600 | 21.9 " | 5.8 | 110.0 | 41.9 " | 197.5 |
| " | 21-6-48 | 6.5 | 1670 | 33.4 " | 6.9 | 1165 | 1.3 " | 5.9 | 82.0 | 21.4 " | 67.9 |
| " | 28-6-48 | 6.1 | 11520 | 218.9 " | 6.1 | 11310 | 63.3 " | 6.0 | 190.0 | 117.0 " | 82.3 |
| " | 12-8-48 | 6.6 | 6000 | 25.8 " | 6.4 | 5435 | 7.8 " | 6.7 | 163.0 | 1.6 " | 36.4 |
| Birch | 18-5-48 | 7.3 | 1270 | 79.9 (HCl) | 6.0 | 1080 | 33.3 (NaOH) | 8.6 | 70.0 | 48.4 (HCl) | 18.9 |
| " | 25-5-48 | 7.5 | 2890 | 306.9 " | 5.1 | 2330 | 43.0 " | 8.8 | 160.0 | 255.6 " | 69.2 |
| " | 9-6-48 | 7.3 | 6000 | 184.4 " | 5.8 | 6000 | 57.1 " | 8.8 | 236.0 | 257.4 " | 108.6 |
| " | 21-6-48 | 7.4 | 4250 | 229.5 " | 6.8 | 4250 | 9.1 " | 9.4 | 132.0 | 316.8 " | 134.1 |
| " | 28-6-48 | 7.2 | 12900 | 417.9 " | 6.7 | 12540 | 42.7 " | 9.1 | 360.0 | 828.0 " | 187.7 |
| " | 12-8-48 | 7.4 | 6000 | 180.0 " | 4.7 | 5893 | 59.0 " | 9.0 | 107.0 | 402.5 " | 190.5 |

TABLE XXIII CONTINUED

|  |  | Leachate |  |  | Distillate |  |  | Residue |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | Date of Collection | pH | Volume Distilled (ml.) | Total Titratable Acidity or Alkalinity as ml. of 0.01 N | pH | Volume Collected (ml.) | Total Titratable Acidity or Alkalinity as ml. of 0.01 N | pH | Volume (ml.) | Total Titratable Acidity or Alkalinity as ml. of 0.01 N | Percentage Titratable Acidity or Alkalinity Accounted for |
| Maple | 18-5-48 | 5.3 | 3330 | 251.3 (NaOH) | 4.9 | 3330 | 15.2 (NaOH) | 4.0 | 19.0 | 238.3 (NaOH) | 100.8 |
| " | 25-5-48 | 5.0 | 2440 | 315.6 " | 4.5 | 2357 | 136.9 " | 5.4 | 28.0 | 118.3 " | 80.8 |
| " | 9-6-48 | 5.5 | 6000 | 161.3 " | 5.7 | 5880 | 33.0 " | 4.4 | 120.0 | 241.9 " | 170.4 |
| " | 21-6-48 | 5.5 | 1180 | 174.5 " | 6.1 | 1070 | 6.0 " | 4.5 | 83.0 | 272.6 " | 159.6 |
| " | 28-6-48 | 5.2 | 10500 | 940.8 " | 6.5 | 9580 | 32.6 " | 4.3 | 255.0 | 918.0 " | 101.1 |
| " | 12-8-48 | 5.9 | 6000 | 242.4 " | 6.3 | 5878 | 13.2 " | 5.0 | 121.6 | 131.8 " | 59.7 |
| Poplar | 18-5-48 | 6.4 | 2750 | 11.4 (NaOH) | 5.1 | 2685 | 30.4 (NaOH) | 7.3 | 35.5 | 1.3 (HCl) | 255.2 |
| " | 25-5-48 | 7.0 | 1330 |  | 5.6 | 1214 | 15.2 " | 8.2 | 88.0 | 30.8 " |  |
| " | 9-6-48 | 6.4 | 6000 | 27.6 " | 6.1 | 5800 | 18.4 " | 7.6 | 119.0 | 22.5 " |  |
| " | 21-6-48 | 7.0 | 1650 |  | 7.0 | 600 |  | 7.9 | 25.0 | 58.5 " |  |
| " | 28-6-48 | 6.4 | 11700 | 91.7 " | 6.5 | 11465 | 39.0 " | 8.3 | 235.0 | 2.1 " | 40.3 |
| " | 12-8-48 | 7.1 | 6000 | 37.8 (HCl) | 7.6 | 5877 | 15.9 (HCl) | 9.0 | 123.0 | 58.5 " | 197.0 |

leachate may cause much of this variation in the recovery of titratable material. Due to the small volume of the distillation flasks, frequent refillings with leachate were necessary. When one of the four distillation units was stopped for refilling, the vacuum in the other three distillation units was depleted and distillation stopped in them also. As soon as the vacuum was restored, the three warm distillation units resumed distilling again before the fourth unit came up to temperature, and it is possible that volatile acids from the warm distillation units condensed in the cold distillation unit. On this assumption, Table XXIV was prepared to show the average percentage recovery of titratable material for the four species which were distilled simultaneously.

## TABLE XXIV

## AVERAGE RECOVERY OF TITRATABLE MATERIALS AFTER DISTILLATION

| Date | Per cent Recovery |
|---|---|
| 18-5-48 | 117.9 |
| 28-6-48 | 102.9 |
| 12-8-48 | 120.9 |

Only three values are given as poplar could not be included in the average for the other three dates. However, these average values are so much more uniform than the data on individual leachates that it lends some weight to the above hypothesis, and the increase in titratable material after distillation might well be due to constitutional changes in the material with prolonged heating.

The birch and maple leachates, which exhibited the two extremes of pH values, are found to retain a high percentage of the total titratable material in the residue. With the beech and poplar leachates on the other hand, it is found that the total titratable material is often about equally divided between the distillate and residue. The residues are much more concentrated, however, and only the residues were studied for the detection of organic acids.

## 3. Electrodialysis of Leached Materials

In the study of the acidic nature of the leachates, some of the material of each species that was precipitated with lead acetate, and leachates that were concentrated by distillation were subjected to electrodialysis so as to obtain a solution containing only anions.

### a. Electrodialysis of Materials Precipitated with Lead Acetate

In the centre compartment of the electrodialysis apparatus was placed 5 grams of lead precipitate of maple leachate of the autumn of 1947. Sufficient water was added to cover the membranes between all compartments. This material was electrodialysed for a total of ninetyfour hours.

After twelve hours there was no color moving out of the centre compartment, but after thirty hours of electrodialysis a faint yellowish-brown color appeared in the anodic compartment and it was apparent that lead was being deposited on the cathode. The color of the solutions in the anodic compartments became more intense as dialysis proceeded. The changes in the pH of the solutions with time are shown in Table XXV. The titratable acidities of the anodic solutions were determined and are recorded in Table XXVI.

## TABLE XXV

## THE pH OF THE SOLUTIONS DURING ELECTRODIALYSIS

| Time (Hrs.) | Cathodic Cell II | Cathodic Cell I | Centre Cell | Anodic Cell I | Anodic Cell II |
|---|---|---|---|---|---|
| 12 | 11.40 | 10.07 | 4.00 | 3.93 | 3.59 |
| 30 | 11.59 | 9.58 | 3.64 | 3.17 | 2.98 |
| 54 | 11.85 | 8.50 | 3.53 | 3.53 | 2.71 |
| 94 | 12.08 | 9.14 | 3.37 | 3.10 | 2.50 |

Following the dialysis of the original lead precipitate the residual material in the centre compartment was removed and saturated with hydrogen sulphide. This material then was replaced in the electrodialysis apparatus and the anodic and cathodic compartments were filled with distilled water. Electrodialysis was continued for thirty-

## TABLE XXVI

## TITRATABLE ACIDITIES OF ANODIC SOLUTIONS OF LEAD PRECIPITATE OF MAPLE (1947)

| Sample | Duration (Hrs.) | Anodic Solution | pH | Volume (ml.) | Titratable Acidity as ml.of 0.01 N NaOH Per solution | Total |
|---|---|---|---|---|---|---|
| Pb-Precipitate from maple | 94 | I | 3.10 | 105 | 39.42 | |
| | | II | 2.50 | 60 | 63.84 | 103.26 |
| Pb-Precipitate from maple + $H_2S$ | 36 | I | 2.00 | 108 | 175.39 | |
| | | II | 1.40 | 103 | 389.92 | 565.31 |
| " | 12 | I | 2.76 | 107 | 26.36 | |
| | | II | 2.31 | 112 | 56.45 | 82.81 |
| " | 24 | I | 2.87 | 118 | 23.79 | |
| | | II | 2.52 | 106 | 77.17 | 100.96 |

six hours. Color appeared in the anodic compartment next to the centre compartment within one hour. The titratable acidities of the anodic solutions (Table XXVI) showed that there was a much greater movement of materials after than before the material was treated with hydrogen sulphide. The anodic solutions were removed after thirty six hours and were replaced with distilled water. After twelve hours these solutions were also removed, and the electrodialysis was continued for another twenty four hours. From the data on titratable acidities it is seen that the acids which dialysed in the first thirty six hours amount to 75 per cent of the total acidity that dialysed in seventy two hours. The treatment with hydrogen sulphide caused a seven fold increase in the amount of dialysable acidic materials.

It was felt that the titratable acidities of the anodic solutions resulting from hydrogen sulphide treatment might be due in part to the hydrogen sulphide present, although only a faint odor could be detected. Accordingly these solutions were heated to boiling to drive off any hydrogen sulphide present, and after filtering off free sulphur the titratable acidities were again determined. The values, however, were the same as before, which indicates that the hydrogen sulphide had no measurable effect on the titratable acidity values, and that the acidic materials present were of a non-volatile nature.

A sample (5g.) of lead precipitate of poplar leachate of the autumn of 1947 in distilled water was electrodialysed after saturating the suspension with hydrogen sulphide, and heating to boiling to drive off any free hydrogen sulphide. Table XXVII contains the data on titratable acidities for various durations of electrodialysis.

The titratable acidity in the second twelve hour run was higher than in the first which, from previous work with maple, would not have been expected. This is believed to be due to a temperature rise to 42°C. following stoppage of the flow of water used to keep the apparatus cool. When this was remedied the temperature came down to normal (23°C). Also the amount of electric current passing through the apparatus was 58 milliamperes at 42°C but dropped to 41 milliamperes when the temperature reached 23°C. This increased amperage would indicate a larger movement of ionic materials.

## TABLE XXVII

## TITRATABLE ACIDITIES OF ANODIC SOLUTIONS OF LEAD PRECIPITATE OF POPLAR (1947)

| Sample | Duration (Hrs.) | Anodic Solution | pH | Volume (ml.) | Titratable Acidity as ml. of 0.01 N NaOH Per Solution | Total |
|---|---|---|---|---|---|---|
| Pb-precipitate from poplar + $H_2S$ | 12 | I | 2.52 | 120 | 90.72 | |
| | | II | 2.12 | 106 | 111.59 | 202.31 |
| " | 12 | I | 2.52 | 120 | 94.08 | |
| | | II | 2.10 | 110 | 135.52 | 229.60 |
| " | 12 | I | 3.09 | 120 | 68.28 | |
| | | II | 2.58 | 105 | 152.88 | 221.16 |

B.B. Coldwell, who also worked with the materials precipitated from the leachates by lead acetate, removed the lead before carrying out his study of these materials. The removal of lead was accomplished by treating with sulphuric acid, and after the lead sulphate was centrifuged off, the reddish-brown supernatant solutions were electrodialysed for twenty four hours. This arbitrary time limit was found to be very convenient and the anodic solutions contained a sufficient amount of dialysable acids for further study. There were no titratable acidity determinations made on these solutions as excess sulphuric acid present would mask the acids originating in the leaf leachates.

During the electrodialysis of leached materials there was very little color movement into the cathodic compartments, but the anodic solutions always contained some color varying from yellow to a deep reddish-brown. During the electrodialysis there was frequently a movement of water into the centre cell, which sometimes necessitated the removal of some of the material from the centre compartment to prevent loss by overflowing. This was especially pronounced in the electrodialysis of the lead precipitated materials that were treated with sulphuric acid.

### b. Electrodialysis of Leachates Concentrated by Distillation

For the investigation of low molecular weight organic acids it was felt that possibly the most profitable line of attack would be the electrodialysis of the leachates which were concentrated by distillation. Accordingly these concentrated leachates were electrodialysed for twenty four hours, and the titratable acidities were determined on the solutions from the anodic and central compartments; the data are contained in Tables XXVIII, XXIX, XXX, XXXI.

## TABLE XXVIII

## ELECTRODIALYSIS OF CONCENTRATED LEACHATES OF MAY 18, 1948

| Species | Solution | pH | Volume (ml.) | Total Titratable Acidity as ml. of 0.01 N NaOH |
|---|---|---|---|---|
| Beech | Anodic I | 3.28 | 125 | 22.40 |
| | Anodic II | 2.20 | 98 | 112.50 |
| | Centre | 2.88 | 250 | 75.60 |
| Birch | Anodic I | 2.87 | 120 | 68.54 |
| | Anodic II | 1.88 | 100 | 296.80 |
| | Centre | 2.90 | 242 | 112.48 |
| Maple | Anodic I | 2.63 | 115 | 115.92 |
| | Anodic II | 1.72 | 103 | 358.45 |
| | Centre | 3.05 | 240 | 282.24 |
| Poplar | Anodic I | 2.86 | 123 | 54.37 |
| | Anodic II | 2.10 | 96 | 116.64 |
| | Centre | 2.95 | 250 | 75.60 |

## TABLE XXIX

## ELECTRODIALYSIS OF CONCENTRATED LEACHATES OF MAY 25, 1948

| Species | Solution | pH | Volume (ml.) | Total Titratable Acidity as ml. of 0.01 N NaOH |
|---|---|---|---|---|
| Beech | Anodic I | 2.90 | 125 | 28.00 |
| | Anodic II | 1.90 | 104 | 121.68 |
| | Centre | 2.75 | 260 | 77.22 |
| Birch | Anodic I | 1.76 | 51 | 25.29 |
| | Anodic II | 1.12 | 87 | 76.38 |
| | Centre | 2.70 | 275 | 360.25 |
| Maple | Anodic I | 2.52 | 117 | 105.53 |
| | Anodic II | 1.65 | 91 | 313.40 |
| | Centre | 2.40 | 236 | 274.70 |
| Poplar | Anodic I | 2.48 | 118 | 52.39 |
| | Anodic II | 1.71 | 105 | 188.48 |
| | Centre | 2.84 | 222 | 53.72 |

## TABLE XXX

## ELECTRODIALYSIS OF CONCENTRATED LEACHATES OF JUNE 28, 1948

| Species | Solution | pH | Volume (ml.) | Total Titratable Material as ml. of 0.01 N NaOH or HCl |
|---|---|---|---|---|
| Beech | Anodic I | 2.50 | 120 | 117.66 |
| | Anodic II | 1.72 | 121 | 513.04 |
| | Centre | 2.75 | 240 | 255.60 |
| | Cathodic I | 9.30 | 125 | 12.13 |
| | Cathodic II | 12.50 | 135 | 696.19 |
| Birch | Anodic I | 3.25 | 60 | 199.68 |
| | Anodic II | 2.50 | 80 | 708.08 |
| | Centre | 2.65 | 265 | 563.71 |
| | Cathodic I | 11.50 | 96 | 50.80 |
| | Cathodic II | 12.80 | 108 | 1180.98 |
| Birch | Anodic I | 1.90 | 63 | 304.54 |
| | Anodic II | 1.35 | 100 | 1150.00 |
| | Centre | 2.40 | 413 | 1519.01 |
| | Cathodic I | 8.80 | 130 | 24.44 |
| | Cathodic II | 12.80 | 117 | 2220.66 |
| Maple | Anodic I | 2.10 | 113 | 358.21 |
| | Anodic II | 1.60 | 112 | 912.95 |
| | Centre | 2.18 | 325 | 1446.90 |
| | Cathodic I | 7.50 | 135 | 5.67 |
| | Cathodic II | 12.50 | 85 | 604.69 |
| Poplar | Anodic I | 2.00 | 82 | 225.99 |
| | Anodic II | 1.50 | 120 | 664.62 |
| | Centre | 2.65 | 255 | 356.79 |
| | Cathodic I | 10.05 | 132 | 82.37 |
| | Cathodic II | 12.60 | 107 | 712.19 |

## TABLE XXXI

## ELECTRODIALYSIS OF CONCENTRATED LEACHATES OF AUGUST 12, 1948

| Species | Solution | pH | Volume (ml.) | Total Titratable Material as ml.of 0.01 N NaOH or HCl |
|---|---|---|---|---|
| Beech | Anodic I | 2.35 | 136 | 109.62 |
| | Anodic II | 2.15 | 92 | 111.14 |
| | Centre | 3.50 | 260 | 71.76 |
| | Cathodic I | 11.30 | 118 | 87.08 |
| | Cathodic II | 11.90 | 107 | 122.41 |
| Birch | Anodic I | 2.50 | 46 | 84.82 |
| | Anodic II | 1.40 | 72 | 746.35 |
| | Centre | 2.75 | 242 | 333.48 |
| | Cathodic I | 8.75 | 126 | 47.12 |
| | Cathodic II | 10.90 | 116 | 661.20 |
| Maple | Anodic I | 2.55 | 122 | 147.38 |
| | Anodic II | 1.70 | 69 | 584.74 |
| | Centre | 2.50 | 257 | 591.89 |
| | Cathodic I | 8.15 | 131 | 5.50 |
| | Cathodic II | 12.50 | 113 | 564.10 |
| Poplar | Anodic I | 3.30 | 50 | 15.90 |
| | Anodic II | 2.00 | 71 | 195.68 |
| | Centre | 3.05 | 240 | 76.32 |
| | Cathodic I | 9.30 | 90 | 48.60 |
| | Cathodic II | 12.20 | 103 | 317.03 |

It is quite characteristic that the anodic solution in the compartment which contained the electrode was always much more acidic than the solution in the anodic compartment next to the centre. It is also noted that after twenty four hours of electrodialysis the reaction of the material in the centre compartment becomes more acidic as shown by both pH and titratable acidity determinations. It would seem then that in the material that is non-dialysable the acidic compounds are much in excess of the basic compounds.

The titratable acidity of the non-dialysable materials has been calculated on a percentage basis of the total titratable acidity found in the centre and anodic compartments. Table XXXII presents the per cent of titratable acidity that is non-dialysable in twenty four hours.

## TABLE XXXII

## NON-DIALYSABLE ACIDITY OF LEACHATES AS PER CENT OF TOTAL TITRATABLE ACIDITY AFTER ELECTRODIALYSIS

| Date | Beech | Birch | Maple | Poplar |
|---|---|---|---|---|
| 18-5-48 | 35.9 | 23.5 | 47.4 | 32.6 |
| 25-5-48 | 34.0 | - | 39.6 | 18.2 |
| 28-6-48 | 28.8 | 44.7 | 53.3 | 28.6 |
| 12-8-48 | 24.5 | 28.7 | 44.7 | 26.5 |
| Average | 30.8 | 32.3 | 46.3 | 26.5 |

While there are large variations within the species themselves, the values would seem to indicate that maple has a higher non-dialysable acidic content than the other species.

Table XXXIII shows the change in the net titratable material in the leachates after electrodialysis. All these leachates become more acidic on electrodialysis except the poplar leachates of August 12. However, during the electrodialysis of the poplar leachate some of the anodic solution was lost from the apparatus which likely can account for this slight change to decreased acidity.

## TABLE XXXIII

### NET TITRATABLE MATERIAL BEFORE AND AFTER ELECTRODIALYSIS (ML. OF 0.01 N)

| Species | Solution | June 28, 1948 | August 12, 1948 |
|---|---|---|---|
| Beech | Concentrated | 117.04 (NaOH) | 1.54 (NaOH) |
| | Electrodialysed | 177.98 (NaOH) | 983.03 (NaOH) |
| Birch | Concentrated | 828.00 (HCl) | 308.50 (NaOH) |
| | Electrodialysed | 888.14 (NaOH) | 456.33 (NaOH) |
| Maple | Concentrated | 918.00 (NaOH) | 203.30 (NaOH) |
| | Electrodialysed | 2107.70 (NaOH) | 754.41 (NaOH) |
| Poplar | Concentrated | 2.05 (HCl) | 47.60 (HCl) |
| | Electrodialysed | 452.84 (NaOH) | 77.73 (HCl) |

When the ratio of the ash content and the titratable acidity are calculated for the electrodialysed concentrated leachates there is an indication that there is a correlation within species between the ash content and the titratable acidity as shown by the relatively constant values obtained from each species in Table XXXIV. Maple has the lowest ash content per liter of 0.01 N titratable acidity, beech and poplar have roughly equal amounts, while birch has the largest amount of ash when calculated on this basis. These values run in the order that would be expected from the data given on page 57, where the acidity entering the soil as pounds of calcium carbonate per acre are given.

## TABLE XXXIV

### TOTAL TITRATABLE ACIDITY AND ASH CONTENT OF ELECTRODIALYSED LEACHATES

| Species | Date | Total Acidity as ml. of 0.01 N NaOH | Grams Ash per Vol. Concentrated | Grams Ash Present with Equivalent of 1L. of 0.01 N Acid |
|---|---|---|---|---|
| Beech | 18-5-48 | 210.50 | 0.1770 | 0.84 |
| " | 25-5-48 | 226.90 | 0.1950 | 0.86 |
| " | 28-6-48 | 886.30 | 1.3823 | 1.56 |
| " | 12-8-48 | 392.52 | 0.3120 | 0.79 |
| Birch | 18-5-48 | 477.82 | 0.5982 | 1.25 |
| " | 25-5-48 | - | 1.8354 | - |
| " | 28-6-48 | 4365.03 | 5.6380 | 1.29 |
| " | 12-8-48 | 1164.65 | 1.5120 | 1.29 |
| Maple | 18-5-48 | 756.61 | 0.3880 | 0.51 |
| " | 25-5-48 | 693.63 | 0.3245 | 0.47 |
| " | 28-6-48 | 2718.06 | 1.3554 | 0.50 |
| " | 12-8-48 | 1324.01 | 0.7020 | 0.53 |
| Poplar | 18-5-48 | 246.61 | 0.3055 | 1.24 |
| " | 25-5-48 | 294.59 | 0.1955 | 0.67 |
| " | 28-6-48 | 1247.40 | 1.0886 | 0.87 |
| " | 12-8-48 | 287.90 | 0.3240 | 1.13 |

The relatively constant ratio obtained within species probably indicates that each species has a definite breakdown ratio of ash and acidic materials. It is likely this ratio will change to higher values as time goes by since the leachates have progressively higher pH values throughout the decomposition period.

## 4. Partition Chromatographic Analysis

The samples of acidic material that were finally analysed by partition chromatography, according to the procedure given in the section on experimental methods, were obtained from the leachates by a preliminary extraction employing a column technique. Only one solution obtained by this preliminary extraction was from a concentrated leachate, while the others were from the anodic solution of electrodialysed concentrated leachates.

The procedure used for this extraction was as follows: The solution was evaporated to small volume (5-10 ml.) on a steam bath and to this small volume was added sufficient silica gel to give a superficially dry powder when thoroughly mixed. Usually 5 to 10 g. of gel were required. 50 per cent n-butanol-chloroform (v/v) was added and the mixture transferred to a glass tube (diameter 5.2 cm., length 20 cm., containing a perforated porcelain disc at the bottom covered with filter paper) and the filtrate collected. The column was allowed to run dry and then it was kept full with 50 per cent n-butanol-chloroform until 500 ml. of filtrate were collected. To the filtrate about 25 ml. of distilled water were added and titrated with 0.1 N sodium hydroxide until the aqueous layer was at pH 9.4. The aqueous layer, plus two washings of the n-butanol-chloroform were collected from a separatory funnel. This aqueous solution of the sodium salts of acids was evaporated to dryness in a vacuum oven at a temperature not exceeding 50°C. The calculated amount of 2 N sulphuric acid required to liberate the acids was added to the residue. The organic acids were then taken up in a few ml. of 50 per cent (v/v) tert-amyl alcohol-chloroform. This solution of the organic acids was then analysed by partition chromatography.

The first partition chromatographic separation of organic acids from leachate material that gave definite results was made on the concentrated maple leachate of the autumn of 1947. The supernatant solution, which was obtained by centrifuging this concentrate to free it of any residue, was extracted with 50 per cent n-butanol-chloroform and the extracted acids were taken up in 16 ml. of 50 per cent tert-amyl alcohol-chloroform. A 4 ml. aliquot was added to a 4 gm. silica gel partition chromatogram and the acid bands were revealed by using the external indicator technique. The total volume of eluate and that of each band were recorded. These acidic bands were collected in separate flasks and titrated with 0.01 N barium hydroxide, which showed an appreciable amount of acidic material in some of the fractions. Two more identical partition columns were run, during which the indicator was not used, but fractions were collected in accordance with the results of the preceeding run with indicator. The similar fractions of these last two runs were combined and titrated with 0.01 N barium hydroxide. Finally the fractions were analysed by filter paper partition chromatography.

The data of the chromatographic analysis of this sample from maple leachate are given in Table XXXV.

TABLE XXXV

FRACTIONS OBTAINED FROM CONCENTRATED MAPLE LEACHATE (1947)

| Fraction | Per cent solvent | Vol. of 0.01 N Barium Hydroxide (ml.) | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 12.75 | - | - | - |
| 2 | 10 | 4.75 | - | - | - |
| 3 | 10-20 | 1.00 | - | - | - |
| 4 | 20 | 6.50 | - | - | - |
| 5 | 35 | 3.50 | - | - | - |
| 6 | 35 | 9.20 | 0.45 | Malic | 5 |
| 7 | 35 | 8.00 | 0.39 | Citric | 3 |
| 8 | 35 | 1.50 | - | - | - |

The analysis of fractions 6 and 7 was repeated on filter paper chromatograms in the following manner: The acid fraction and also a known acid solution were placed on the same spot at the bottom of the paper. Care was taken to see that the solution which was first applied to the paper had become dry before the second solution was applied to the same spot. This was done to keep the area of the spot as small as possible and consequently to give a more distinct and intense acid spot when the chromatogram was sprayed with indicator. The acids so spotted are given in Table XXXVI with the resulting $R_f$ values. From this analysis it is seen that the unknown fractions give $R_f$ values identical with those for malic and citric acids.

TABLE XXXVI

$R_f$ VALUES OF ACIDIC FRACTIONS AND KNOWN ACIDS

| Sample in Spot | $R_f$ Value |
|---|---|
| Malic acid and fraction 6 | 0.39 |
| Malic acid | 0.39 |
| Fraction 6 | 0.39 |
| Citric acid | 0.32 |
| Citric acid and fraction 7 | 0.32 |
| Fraction 7 | 0.32 |
| Fractions 6 and 7 | 0.31 and 0.39 |

The anodic solutions obtained from the hydrogen sulphide treated lead precipitated organic matter of maple leachate was analysed in a similar manner, except that at this point the collection of successive 50 ml. portions of the eluate from the silica column was introduced.

In Table XXXVII it is seen that malic and citric acids are present in this sample, and both acids are found in the same fraction. Oxalic acid is also present, and is easily identified by its comet shaped spot of low $R_f$ value. The lead acetate is apparently capable of precipitating these organic acids from the leachate.

TABLE XXXVII

FRACTIONS OBTAINED FROM LEAD PRECIPITATE OF MAPLE WHICH WAS DECOMPOSED WITH HYDROGEN SULPHIDE

| Fraction | Per cent Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 5 | 9.50 | - | | |
| 2 | 5 | 1.75 | - | | |
| 3 | 5 | 4.00 | - | | |
| 4 | 10 | 7.10 | - | | |
| 5 | 10 | 3.00 | - | | |
| 6 | 20 | 7.20 | Comet shape | Oxalic | 7.0 |
| 7 | 20 | 5.60 | " " | | |
| 8 | 20 | 3.10 | - | | |
| 9 | 35 | 2.90 | - | | |
| 10 | 35 | 5.40 | 0.43<br>0.31 | Malic<br>Citric | 3.0 |
| 11 | 35 | 5.25 | - | | |
| 12 | 35 | 3.80 | - | | |

In the chromatographic analysis of this sample a total of 25 fractions were collected, but no evidence of organic acids in the last 15 fractions could be obtained. The last 10 fractions did contain titratable acidity in appreciable amounts but this turned out to be sulphuric acid which was evidently leached from the column.

The anodic solutions obtained from the hydrogen sulphide treated lead precipitated organic matter of poplar leachate was also analysed and the data are present in Table XXXVIII.

The $R_f$ value of an acid in fraction 1 is similar to that of malic acid. The partition coefficient of malic acid is such that it should not be found in the first fraction, and it is probable that the occurance of malic acid in the first fraction was due to the addition of too large a volume of the acid solution with the result that a small amount of malic acid immediately was carried through the column.

The $R_f$ values of fractions 12 and 13 are seen to differ somewhat from the $R_f$ value of fraction 14, although all three fractions have been identified as citric acid. This discrepancy is due to the analysis being performed on two different filter paper sheets. Fractions 12 and 13 were analysed on one sheet of filter paper, while fraction 14 was analysed on another sheet. However on each sheet the $R_f$ values of the fractions were identical with the $R_f$ value of citric acid which was spotted on the

## TABLE XXXVIII

### FRACTIONS OBTAINED FROM LEAD PRECIPITATE OF POPLAR WHICH WAS DECOMPOSED WITH HYDROGEN SULPHIDE

| Fraction | Per cent Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 5 | 22.90 | 0.83<br>0.42 | Malic | - |
| 2 | 5 | 5.10 | 0.83 | - | - |
| 3 | 5 | 4.50 | - | - | - |
| 4 | 5 | 2.55 | - | - | - |
| 5 | 10 | 5.50 | - | - | - |
| 6 | 10 | 4.00 | - | - | - |
| 7 | 10 | 4.80 | - | - | - |
| 8 | 20 | 15.20 | Comet shape | Oxalic | 9.0 |
| 9 | 20 | 3.95 | - | - | - |
| 10 | 20 | 4.00 | - | - | - |
| 11 | 35 | 27.50 | 0.42 | Malic | 17.0 |
| 12 | 35 | 21.80 | 0.31 | Citric | 57.0 |
| 13 | 35 | 60.00 | 0.31 | Citric | |
| 14 | 35 | 11.40 | 0.37 | Citric | |

sheets also. This variation of $R_f$ values of an acid which is spotted on different sheets is probably due to a difference in the moisture content of the sheets.

In this analysis several more fractions were collected than are recorded in Table XXXVIII. The use of 70 per cent n-butanol-chloroform was also tried but it was of no value and in these later fractions sulphuric acid was leached from the column.

The tables in the appendix contain the results of the chromatographic analysis of the lead precipitates that were decomposed with sulphuric acid and of the leachates concentrated by distillation. In general only fifteen fractions at the most are recorded although more were collected. The analysis of the later fractions revealed no organic acids and therefore have been omitted from the tables.

Table XXXIX gives a summary of the organic acids that were identified in all the analyses. The various samples of leached material listed in the table that are followed by "$H_2S$" or "$H_2SO_4$" were decomposed with that reagent before electrodialysis. The acids are reported in milligram amounts. These amounts have been calculated from the titration values of the fractions, after correcting for blank titrations. The majority of the fractions contained varying amounts of colored materials, and since electrodialysis has shown the colored materials to be acidic in nature the amounts quoted for the various acids probably are somewhat too high. The first fraction of each analysis was deeply colored and the color gradually decreased in the succeeding fractions. However it was noted that fractions of high titratable acidity and which were shown to contain an organic acid were more highly colored than were fractions of lower titratable acidity. The fractions from autumn leachates showed this phenomenon to a greater extent than the more highly colored fractions from the summer leachates.

### TABLE XXXIX

### ACIDS OBTAINED FROM VARIOUS LEACHATES

### (MG. PER LITER OF LEACHATE)

| Sample | Oxalic | Malic | Citric |
|---|---|---|---|
| Beech Lead Ppt. ($H_2SO_4$) | - | - | - |
| " Conc. 25-5-48 | - | - | - |
| " " 28-6-48 | - | - | - |
| " " 12-8-48 | 0.33 | - | - |
| Birch Lead Ppt. ($H_2SO_4$) | 0.19 | 0.03 | 0.16 |
| " Conc. 25-5-48 | 3.46 | trace | trace |
| " " 28-6-48 | 0.78 | 0.46 | - |
| " " 12-8-48 | 2.83 | 0.50 | - |
| Maple Conc. Autumn 1947 | - | 2.91 | 1.16 |
| " Lead Ppt. ($H_2S$) | 0.74 | 0.32# | |
| " " " ($H_2SO_4$) | 0.59 | 0.24 | 0.49 |
| " Conc. 25-5-48 | 6.15 | 1.64 | 1.23 |
| " " 28-6-48 | 0.86 | 0.19 | 0.29 |
| " " 12-8-48 | 0.30 | - | - |
| Poplar Lead Ppt. ($H_2S$) | 0.38 | 0.72 | 2.40 |
| " " " ($H_2SO_4$) | 0.55 | 0.55 | 1.80 |
| " Conc. 25-5-48 | - | - | - |
| " " 28-6-48 | - | - | - |
| " " 12-8-48 | 0.17 | 0.17 | - |

# - Both malic and citric acids present in one fraction; total amount 0.32 mg. per liter of leachate.

## 5. Buffer-Index Curves

Buffer-index curves were determined for the acid fractions, obtained from the leachates by silica gel chromatographic separations, which gave distinct acid spots in the filter-paper chromatographic analyses. These fractions which were shown to contain citric, malic, and oxalic acids by filter-paper chromatograms were treated with Norit-A to remove the color present. In addition the acidic fraction giving a spot or spots of high $R_f$ value (similar to succinic or malonic acids) was also titrated to obtain a buffer-index curve.

An aqueous solution of each such fraction was titrated with aliquots of 0.01 N potassium hydroxide and the subsequent pH changes were noted.

Buffer-index curves were also determined for the C.P. acids and they checked well with those in the literature. The value of the buffer-index of a given acid at various pH values is dependent upon the concentration of the solution.

Citric Acid. The buffer-index curve for a known sample of citric acid is given in Fig. 4 along with the buffer-index curve for the acid in fraction 10, Table XLIV, which gave an $R_f$ value of 0.31. This fraction was practically free of colored material and on treatment with Norit - A a colorless solution resulted. When the solution was evaporated to small volume a white solid was obtained.

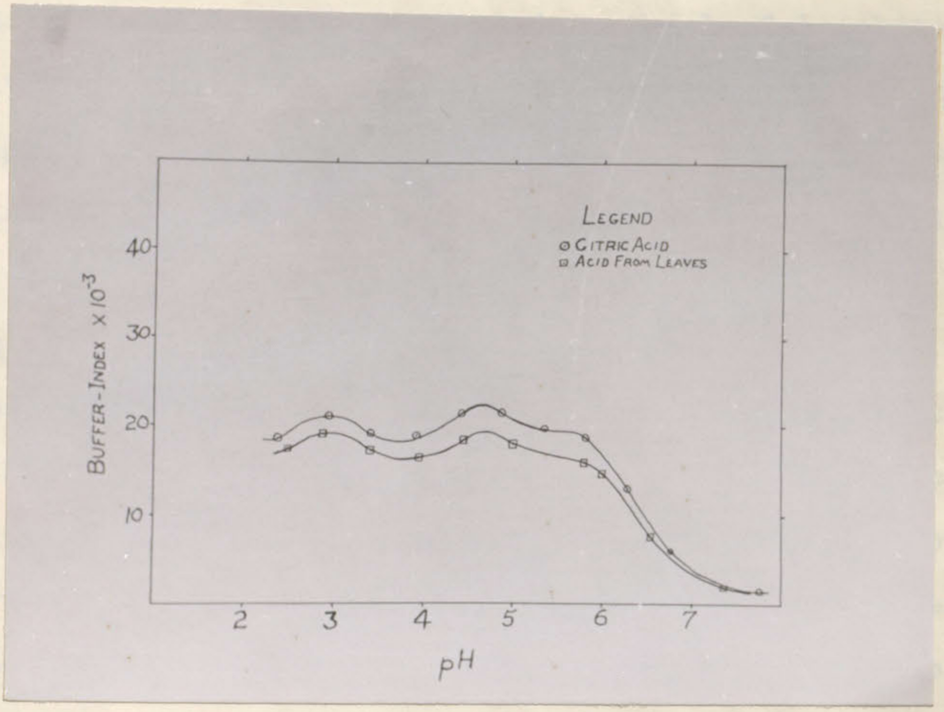

Fig. 4. Citric acid buffer-index curve.

The dry solid weighed 35 mg. and was dissolved in 6 ml. of boiled distilled water for the titration procedure. Then as a comparison 35 mg. of C.P. citric was similarly treated. The general shape of the curves and the practically identical points of maximum buffering strongly indicate that the acid obtained from the leaf leachate is citric acid. The fact that the C.P. citric acid has a slightly higher buffering capacity than the acid from the leachate suggests that the latter is not absolutely pure.

Malic Acid. Most of the fractions which filter-paper chromatographic analyses indicated to contain malic acid were highly colored. Charcoal failed to remove this color completely, and as several treatments were performed on the fractions the content of malic acid was also decreased. The buffer-index curve was determined on a sample consisting of a combination of several fractions ($R_f$ value of 0.40 - 0.45). The similarity of the buffer-index curves (Fig. 5) for the acid in this sample and C.P. malic acid, both of which drop at pH 5.0, is additional evidence that malic acid occurs in some leachates. The buffer-index for 10 mg. of C.P. malic acid in 6 ml. of water is double that of the acid from the leaves. Then approximately 5.0 mg. are present in the sample indicating that much of the acid was lost during the Norit-A treatments.

Oxalic Acid. In Fig. 6 three buffer-index curves are given. The curve obtained for 35 mg. of C.P. oxalic acid

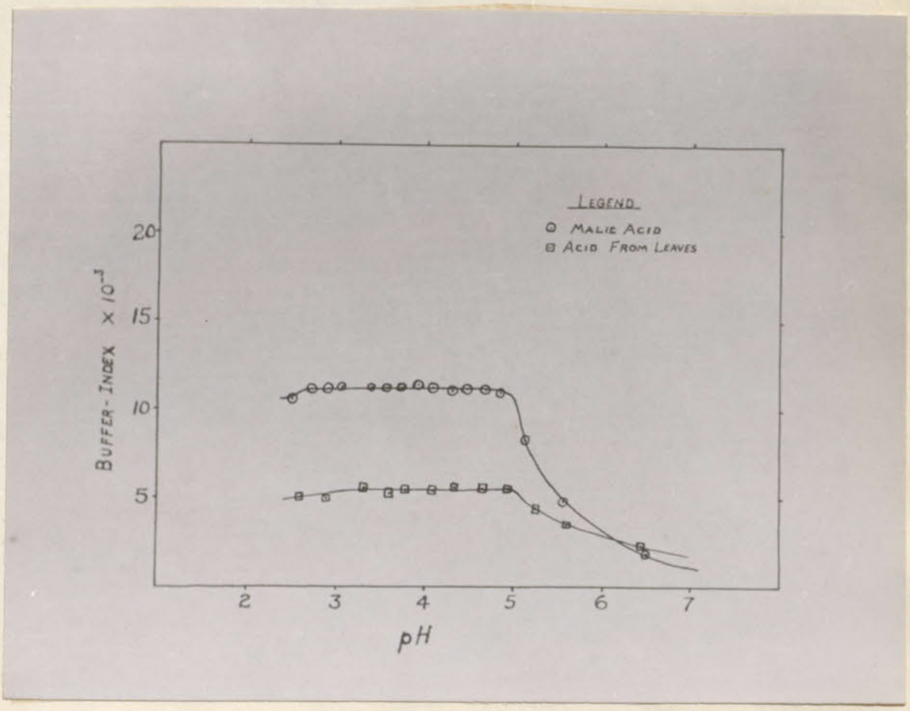

Fig. 5. Malic acid buffer-index curve.

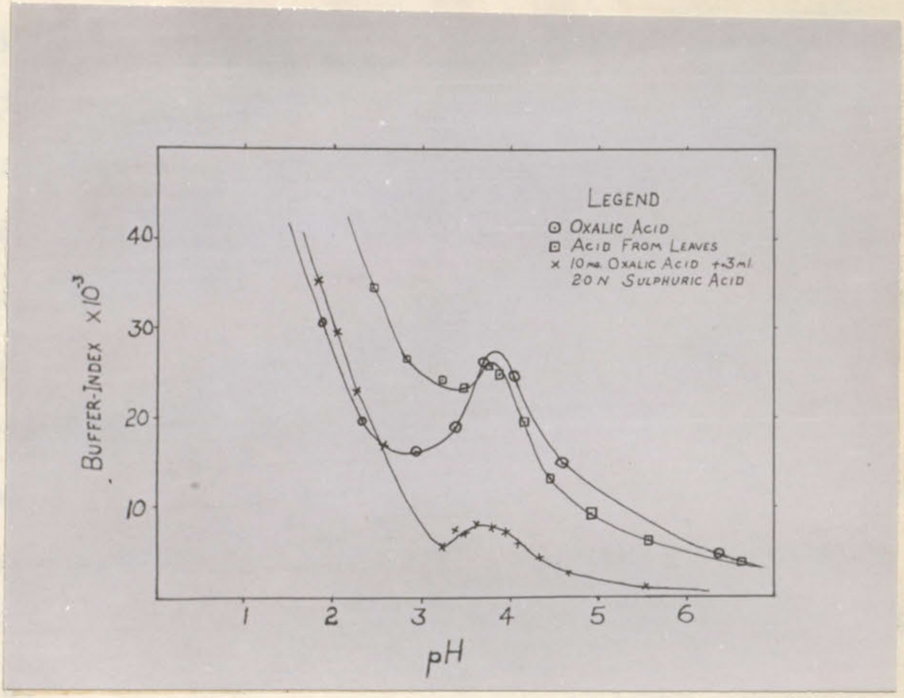

Fig. 6. Oxalic acid buffer-index curve.

in 6 ml. of water gives a peak at about pH 3.8. When the curve for the acid obtained from several fractions, which gave comet shaped spots of low $R_f$ value on filter paper, is drawn evidence for the presence of oxalic acid is given by a peak at pH 3.8. It is to be noted, however, that the large depression for C.P. oxalic acid at pH 2.9 is not present in this curve, but a small depression is found at pH 3.5. The third curve was obtained from a 6 ml. solution containing 10 mg. of oxalic acid and 0.3 ml. of 2.0 N sulphuric acid. In this curve it is seen that the depression has been changed to pH 3.2. It is probable that the colored material not removed by Norit-A has a buffering capacity around pH 3.0 and therefore the large depression characteristic of oxalic acid is obscured. These curves indicate that oxalic acid is present in leaf leachates.

Several of the acidic fractions giving an $R_f$ value of 0.65 - 0.90 on filter paper chromatograms were combined and treated with Norit-A, but much of the color remained. The buffer-index curve for this sample is given in Fig. 7. The buffer-index curves for succinic and malonic acids also are given since their $R_f$ values are of the same order. The curve for the acidic fraction from the leaves shows that an acid that buffers between the pH values of 3.0 and 4.0 is present. The slight bump in this curve at pH 5.0 suggests that malonic or succinic acid, or both, which buffer strongly at pH 5.0, may be present also.

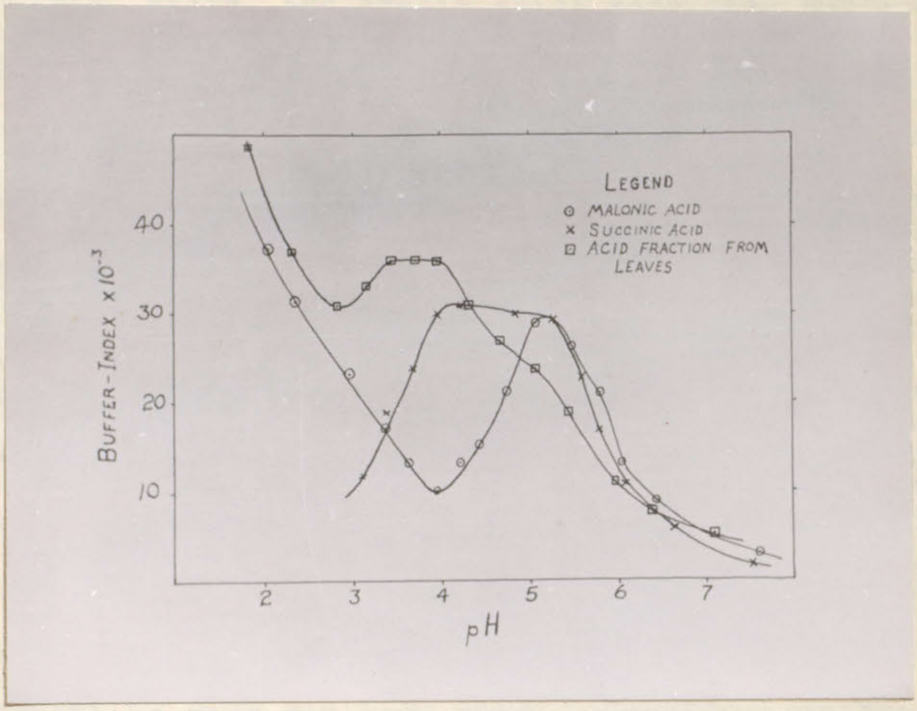

Fig. 7. Buffer-index curves of succinic and malonic acids, and acidic fraction from leaves.

## 6. Spot Tests

Qualitative chemical tests were applied to some of the acidic fractions obtained from the silica gel chromatograms. Fractions which filter paper analyses indicated to be citric, malic, and oxalic acids were then subjected to tests for these acids as outlined by Feigl (29).

These tests depend upon the development of color or fluorescence and it was found that the color present in the fractions interfered with or obscured the colors obtained from the chemical reactions of the tests. This color contamination could be almost wholly removed from the fractions of autumn leachates by heating with Norit-A and filtering through sintered glass micro-filters. The color in the fractions obtained from summer leachates was more difficult to remove and the results of the tests on these fractions were not very good. However since the filter paper method was capable of identifying oxalic, malic, and citric acids in the highly colored fractions, these spot tests on the autumn leachates are sufficient to confirm the validity of the filter paper method.

Citric Acid. The ammonium salt of citracinic acid has a deep blue fluorescence. Citric acid can be readily converted to citracinic acid (2, 6-dihydroxy-4-pyridinecarboxylic acid) by the following method: A drop of the solution to be tested is evaporated to dryness in a microcrucible and the residue treated with 4 drops of thionyl chloride and fumed; about 8 drops of concentrated aqueous ammonia are added and the mixture boiled over a microburner until about 2 drops of liquid remain in the crucible. On cooling 6 drops of concentrated sulphuric acid are added and heating is continued until sulphuric acid vapor is given off. The contents of the crucible are poured into a test tube and rendered ammoniacal, when the solution fluoresces if citric acid is present. The fluorescence is visible by daylight when large amounts of citric acid are present, otherwise a source of ultraviolet light is necessary. The test is specific for citric and aconitic acids.

The results of this procedure on the solutions listed in Table XL indicate that the fractions whose acid gives an Rf value of 0.31 -0.38 on filter paper contain citric acid. According to Isherwood trans-aconitic acid would leave the silica column before malic or citric acids.

Malic Acid. When concentrated sulphuric acid containing a little B-naphthol is heated in the presence of malic acid a blue fluorescence appears. Other acids do not interfere in the reaction but hydroxy acids such as glycollic acid and tartaric acid cause a more or less deep green fluorescence.

The test is performed in the following manner: A drop of the solution to be tested is put in a small test tube and a 1.5 ml. aliquot of a solution of B-naphthol in concentrated sulphuric acid (0.0025 g. B-naphthol per 100 ml.)is added. The solution is heated on a water bath for some time and in the presence of malic acid a yellow color with a blue fluorescence appears. If only a small amount of malic acid is present the yellow color is not noticeable, but the fluorescence is easily seen under ultraviolet light.

The results of this procedure on the solutions listed in Table XL indicate that the fractions whose acid gives an $R_f$ value of 0.40 - 0.45 on filter paper contains malic acid.

Oxalic Acid. On melting together oxalic acid and diphenylamine, a triphenylmethane dyestuff is formed, diphenylamine blue or aniline blue. The formation of aniline blue in the reaction with diphenylamine is very generally specific for oxalic acid.

A few ml. of the solution to be tested are evaporated to dryness in a micro-test-tube; a little diphenylamine is added and melted over a free flame. On cooling, the melt is taken up in a drop of alcohol, when a blue color indicates the presence of oxalic acid. The results given in Table XL are additional evidence to indicate that the acid fractions, which give a comet shape of very low $R_f$ value on filter paper, contain oxalic acid.

## TABLE XL

## RESULTS OF SPOT TESTS ON ACID FRACTIONS

| Sample | Test for | | |
|---|---|---|---|
| | Citric Acid | Malic Acid | Oxalic Acid |
| Citric | Blue fluorescence | nil | nil |
| Malic | nil | Blue fluorescence | nil |
| Oxalic | nil | nil | Blue color |
| $R_f$ = 0.31 | Blue fluorescence | nil | nil |
| $R_f$ = 0.43-0.45 | nil | Blue fluorescence | nil |
| Comet shaped spot | nil | nil | Blue color |

# V GENERAL DISCUSSION

In this work evidence has been presented to show that citric, malic, and oxalic acids occur in the leachates of decomposing leaves of some deciduous trees. These leachates are believed to contain a complexity of acidic materials as shown by the intense color of the anodic solutions obtained by electrodialysis, and from the high content (20 - 25 per cent) of non-dialysable acidity. The movement of the isolated low molecular weight organic acids on silica gel chromatograms and on filter paper chromatograms is identical to the movement of the chemically pure reagent acids. In addition the results of buffer-index curves and spot tests are in agreement with the chromatographic results. The fact that these acids have been identified in leaf leachates entering the soil lends some weight to Jones and Willcox hypothesis (3) that hydroxy-acids play a part in the podsolization process.

Oxalic acid was found in the leachates of the four leaf species, but it was found in only one beech leachate which was collected on August 12, 1948. Malic and citric acids were found in the birch, maple, and poplar leachates, but no evidence was obtained that these acids occur in beech leachates. In addition to these three acids an unidentified acidic fraction which gives a high $R_f$ value on filter paper chromatograms has been found in the leachates of the four species. The fact that malic and citric acids were not found in any beech leachates is worthy of note since the beech leaves show the greatest resistance to decomposition as judged by the residues remaining after eleven months exposure.

Citric acid was found in birch, maple, and poplar leachates during the fall, but during the summer it was found only in maple leachates. Malic acid, however, was found in the birch, maple, and poplar leachates of both fall and summer. Oxalic acid was found in the leachates of these three species throughout the period of study. The unidentified acidic fraction giving a high $R_f$ value (0.65 - 0.90) was much more prominent during the summer months and was found in the leachates of all four species.

The identified acids, calculated as per cent of the dialysable acidity after electrodialysis are given in Table XLI. These values must be taken with reserve, however, since the methods used in the analyses cannot be considered quantitative. Taking the maple concentrate of May 25, as an example, the value for oxalic acid is obtained as follows: The titratable acidity of the fractions, collected from the silica gel chromatograms, which contained oxalic acid is equal to 20.4 ml. of 0.01 N barium hydroxide when corrected for the acidity of the solvent (Table XLVII). The titratable acidity of the anodic solutions (Table XXIX) is 418.9 ml. of 0.01 N sodium hydroxide. Therefore oxalic acid may be said to contribute 4.9 per cent of the titratable acidity in the anodic solutions. It is seen from Table XLI that the identified acids constitute a highly variable proportion of the dialysable acidity. As a rough estimate, during an average rain in the fall or early summer, the total amount of these identified acids leached from the leaves into the soil is in the order of 0.02 lbs. per acre for birch, maple, and poplar. This fact, along with the data in Table XLI suggest that malic, citric, and oxalic acids play a very minor role in the podsolization process. The data in Table XLI indicate that the identified acids contribute more to the acidity of birch, maple, and poplar leachates during the fall and early summer. Thus the fall and early summer should be the best time to study these leachates. Beech, however, shows a high resistance to decomposition and but meagre production of low molecular weight acids. For this species no particular time of study can be recommended.

The leachates concentrated by distillation under reduced pressure give almost identical qualitative results as leachates whose materials were precipitated with lead acetate. This is shown by the maple leachates of 1947 on which analyses were performed on the concentrated material and on the lead precipitate. The birch precipitate of the fall gave similar results as the birch concentrates of early summer. (Table XXXIX) It is expected that analysis of the lead precipitates of the summer would give results similar to those obtained on the concentrates. In con-

## TABLE XLI

## ACIDS IDENTIFIED AS PER CENT OF THE TITRATABLE ACIDITY AFTER ELECTRODIALYSIS

| Sample | Oxalic | Malic | Citric | Total |
|---|---|---|---|---|
| Beech Conc. 12-8-48 | 1.5 | - | - | 1.5 |
| Birch Conc. 25-5-48 | 16.5 | - | - | 16.5 |
| " " 28-6-48 | 0.8 | 0.5 | - | 1.3 |
| " " 12-8-48 | 5.4 | 0.6 | - | 6.0 |
| Maple Lead Ppt. ($H_2S$) | 0.9 | 0.6# | | 1.5 |
| " Conc. 25-5-48 | 4.9 | 1.4 | 1.3 | 7.6 |
| " " 28-6-48 | 1.1 | 0.3 | 0.4 | 1.8 |
| " " 12-8-48 | 0.5 | - | - | 0.5 |
| Poplar Lead Ppt. ($H_2S$) | 2.2 | 4.1 | 13.8 | 20.1 |
| " Conc. 28-6-48 | 0.3 | - | - | 0.3 |
| " " 12-8-48 | 1.0 | 1.0 | - | 2.0 |

# - Both malic and citric acids present in one fraction; total per cent is 0.6.

sidering the relative merits of distillation versus precipitation with lead acetate at room temperature, the latter is recommended as the better method for studying the acidic compounds since the effect of prolonged heating during distillation is liable to cause constitutional changes in the leached materials. This fact is borne out by an average increase in titratable materials (Table XXIV). In addition the dried lead precipitates can be readily stored for analysis at a convenient time. The two methods of decomposing the lead precipitates, i.e. with hydrogen sulphide or sulphuric acid are found to give the same qualitative results. However, since hydrogen sulphide is a milder reagent and any excess can be readily removed, it is to be preferred.

The unidentified acidic fractions that move rapidly on silica gel partition chromatograms and whose $R_f$ value on paper partition chromatograms is 0.65 - 0.90 contain highly colored materials. If low molecular weight organic acids are present in these fractions paper chromatography is practically useless for identifying them under the conditions used because the colored materials give acid streaks with high $R_f$ values. Removal of a large part of the color might be accomplished by employing a longer silica gel column, provided the colored materials move appreciably faster than the low molecular weight acids. In the silica gel chromatographic procedures most of the colored materials were removed with about 200 ml. of solvent, so with a longer silica gel column any low molecular weight acids that might be present in the above unidentified fractions possibly would occur in later fractions containing less color. Also a second chromatographic analysis on silica gel of the early fractions might be helpful. During the preliminary work on filter paper partition chromatography solvents other than n-butanol were investigated (Table XI). There it is shown that methyl-amyl alcohol (2-hexanol) gave good results with the acids used, and the $R_f$ values of succinic and malonic acids (0.46 and 0.38 respectively) are much lower than with n-butanol as solvent. Thus methyl-amyl alcohol might be an aid to identifying any low molecular weight acids which give high $R_f$ values in n-butanol, especially when an intense color is present.

For a more quantitative study of the low molecular weight acids occuring in the leachates, analysis of the lead precipitates would probably be the most promising. Lead acetate may not cause complete precipitation of the low molecular weight acids, but if the lead precipitates from the leachates were analysed by the methods outlined in the body of this thesis along with a known solution of acids, also precipitated with lead acetate as a control, a better estimate of the amount of the acids occuring in the leachates could be obtained.

# VI SUMMARY

1. The importance of the acidic nature of the substances entering the soil from the forest litter, in relation to their effect on podsolization has been emphasized.

2. Leaves were collected during the autumn of 1947 from prepared sites under four species of trees and estimates of the leaf fall in pounds per acre for each species have been obtained.

3. The leaves of each species were placed on separate trays in the woods and the leachates resulting from natural rainfall were collected.

4. The pH, titratable acidity or alkalinity, and the ash content were determined on the leachates after each rain, and an estimate of the acidity or alkalinity in terms of pounds of calcium carbonate per acre entering the soil for each species during eleven months has been obtained. During the period of study (Oct. 1947 to Sept. 1948) the leachates of beech, maple, and poplar were acidic but approached neutrality toward the end of this period. Birch leachates were acidic in the fall but from early summer onward they were consistently alkaline.

5. Two methods of concentrating the small amount of materials in the leachates have been described. These are: (a) Precipitation with a solution of neutral lead acetate,

and (b) Distillation under reduced pressure.

6. The concentrates, and the lead precipitates after decomposition with hydrogen sulphide or sulphuric acid, were electrodialysed and thus a separation of acidic from non-acidic materials has been accomplished.

7. Previously described silica gel partition chromatography and filter paper partition chromatography procedures have been modified and applied to the investigation of low molecular weight acids in the leaf leachates.

8. The low molecular weight acids present in the anodic solutions from the electrodialysis procedure have been separated by silica gel partition chromatography. At the most, four distinct fractions were obtained.

9. Filter paper partition chromatography has been used to identify three of the acidic fractions. Citric, malic, and oxalic acids have been shown to be present in several leachates of birch, maple, and poplar, particularly in the fall and early summer. Beech leachates, however, contained none of these acids until late summer, and then only oxalic acid was found.

10. Buffer-index curves and spot tests have been used to confirm the identity of the isolated acids.

11. Improvements in the chromatographic methods have been

suggested so that better separations of the early acidic fractions may be obtained. A more quantitative method for the estimation of the acids in the leachates has been proposed.

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# VIII APPENDIX

## TABLE XLII

## FRACTIONS OBTAINED FROM LEAD PRECIPITATE OF BIRCH WHICH WAS DECOMPOSED WITH SULPHURIC ACID

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 5 | 10.00 | 0.87 | - | - |
| 2 | 5 | 3.10 | - | - | - |
| 3 | 5 | 4.00 | - | - | - |
| 4 | 5 | 3.40 | - | - | - |
| 5 | 10 | 3.40 | - | - | - |
| 6 | 10 | 4.30 | - | - | - |
| 7 | 10 | 4.40 | Comet shape | Oxalic | 13.0 |
| 8 | 20 | 18.40 | Comet shape | Oxalic | |
| 9 | 20 | 4.90 | - | - | - |
| 10 | 20 | 3.95 | - | - | - |
| 11 | 35 | 3.40 | 0.45 | Malic | 2.0 |
| 12 | 35 | 7.00 | 0.38 | Citric | 11.0 |
| 13 | 35 | 13.10 | 0.38 | Citric | |
| 14 | 35 | 13.00 | - | - | - |
| 15 | 35 | 4.00 | - | - | - |

## TABLE XLIII

## FRACTIONS OBTAINED FROM LEAD PRECIPITATE OF MAPLE WHICH WAS DECOMPOSED WITH SULPHURIC ACID

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 27.25 | 0.68 | - | - |
| 2 | 10 | 26.00 | 0.68 | - | - |
| 3 | 10 | 20.00 | 0.67 | - | - |
| 4 | 10 | 11.00 | - | - | - |
| 5 | 10 | 9.0 | - | - | - |
| 6 | 10 | 34.20 | Comet shape | Oxalic | 36.0 |
| 7 | 20 | 24.75 | Comet shape | Oxalic | |
| 8 | 20 | 13.35 | - | - | - |
| 9 | 20 | 10.50 | 0.45 | Malic | 16.0 |
| 10 | 35 | 16.00 | 0.45 | Malic | |
| 11 | 35 | 18.10 | - | - | - |
| 12 | 35 | 27.90 | 0.34 | Citric | 30.0 |
| 13 | 35 | 21.15 | 0.34 | Citric | |
| 14 | 35 | 14.75 | - | - | - |
| 15 | 35 | 9.45 | - | - | - |

## TABLE XLIV

## FRACTIONS OBTAINED FROM LEAD PRECIPITATE OF POPLAR WHICH WAS DECOMPOSED WITH SULPHURIC ACID

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 29.00 | - | - | - |
| 2 | 10 | 7.55 | - | - | - |
| 3 | 10 | 31.60 | Comet shape | Oxalic | 26.0 |
| 4 | 10 | 11.40 | Comet shape | Oxalic | |
| 5 | 20 | 1.95 | - | - | - |
| 6 | 20 | 6.35 | - | - | - |
| 7 | 20 | 17.05 | 0.45 | Malic | 26.0 |
| 8 | 20 | 24.00 | 0.45 | Malic | |
| 9 | 35 | 13.30 | 0.31 | Citric | 82.0 |
| 10 | 35 | 110.20 | 0.31 | Citric | |
| 11 | 35 | 8.45 | 0.31 | Citric | |
| 12 | 35 | 5.45 | - | - | - |

## TABLE XLV

### FRACTIONS OBTAINED FROM BEECH LEACHATE OF MAY 25, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 5.00 | 0.70 | - | - |
| 2 | 10 | 2.30 | 0.70 | - | - |
| 3 | 10 | 1.50 | 0.70 | - | - |
| 4 | 10 | 0.90 | - | - | - |
| 5 | 10 | 0.90 | - | - | - |

## TABLE XLVI

### FRACTIONS OBTAINED FROM BIRCH LEACHATE OF MAY 25, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 17.30 | - | - | - |
| 2 | 10 | 8.75 | 0.72 | - | - |
| 3 | 10 | 3.75 | 0.72 | - | - |
| 4 | 10 | 3.20 | - | - | - |
| 5 | 10 | 6.30 | Comet shape | Oxalic | 10.0 |
| 6 | 20 | 11.75 | Comet shape | Oxalic | |
| 7 | 20 | 4.65 | - | - | - |
| 8 | 20 | 2.60 | - | - | - |
| 9 | 20 | 3.15 | Very faint evidence of malic | | |
| 10 | 35 | 4.25 | - | - | - |
| 11 | 35 | 5.15 | Very faint evidence of malic | | |
| 12 | 35 | 4.10 | - | - | - |

## TABLE XLVII

### FRACTIONS OBTAINED FROM MAPLE LEACHATE OF MAY 25, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 12.80 | - | - | - |
| 2 | 10 | 10.75 | 0.69 | - | - |
| 3 | 10 | 7.15 | 0.69 | - | - |
| 4 | 10 | 4.30 | - | - | - |
| 5 | 10 | 3.62 | - | - | - |
| 6 | 20 | 14.43 | Comet shape | Oxalic | 15.0 |
| 7 | 20 | 7.50 | Comet shape | Oxalic | |
| 8 | 20 | 3.55 | - | - | - |
| 9 | 20 | 3.20 | - | - | - |
| 10 | 35 | 6.95 | 0.44 | Malic | 4.0 |
| 11 | 35 | 4.40 | - | - | - |
| 12 | 35 | 5.65 | 0.33 | Citric | 3.0 |

## TABLE XLVIII

### FRACTIONS OBTAINED FROM POPLAR LEACHATE OF MAY 25, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 9.75 | - | - | - |
| 2 | 10 | 4.50 | 0.73 | - | - |
| 3 | 10 | 1.60 | - | - | - |
| 4 | 10 | 2.35 | - | - | - |

## TABLE XLIX

### FRACTIONS OBTAINED FROM BEECH LEACHATE OF JUNE 28, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 9.20 | - | - | - |
| 2 | 10 | 4.60 | 0.69 | - | - |
| 3 | 10 | 1.90 | - | - | - |
| 4 | 10 | 2.30 | - | - | - |

## TABLE L

### FRACTIONS OBTAINED FROM BIRCH LEACHATE OF JUNE 28, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 56.75 | - | - | - |
| 2 | 10 | 14.05 | 0.70 | - | - |
| 3 | 10 | 15.30 | 0.70 | - | - |
| 4 | 10 | 18.05 | Comet shape | Oxalic | 10.0 |
| 5 | 10 | - | - | - | - |
| 6 | 20 | 7.55 | - | - | - |
| 7 | 20 | 6.60 | 0.43 | Malic | 6.0 |
| 8 | 20 | 5.00 | 0.43 | Malic | |
| 9 | 20 | 2.85 | - | - | - |
| 10 | 35 | 9.55 | - | - | - |
| 11 | 35 | 7.10 | - | - | - |
| 12 | 35 | 4.60 | - | - | - |

## TABLE LI

## FRACTIONS OBTAINED FROM MAPLE LEACHATE OF JUNE 28, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 18.00 | - | - | - |
| 2 | 10 | 8.20 | 0.68 | - | - |
| 3 | 10 | 6.65 | Comet shape | Oxalic | 9.0 |
| 4 | 10 | 9.10 | Comet shape | Oxalic | |
| 5 | 10 | 2.95 | - | - | - |
| 6 | 20 | 5.10 | - | - | - |
| 7 | 20 | 3.70 | 0.40 | Malic | 2.0 |
| 8 | 20 | 2.70 | - | - | - |
| 9 | 20 | 1.95 | - | - | - |
| 10 | 35 | 5.55 | 0.31 | Citric | 3.0 |
| 11 | 35 | 4.40 | - | - | - |
| 12 | 35 | 2.85 | - | - | - |

## TABLE LII

## FRACTIONS OBTAINED FROM POPLAR LEACHATE OF JUNE 28, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 13.50 | - | - | - |
| 2 | 10 | 6.35 | 0.71 | - | - |
| 3 | 10 | 2.80 | - | - | - |
| 4 | 10 | 3.40 | Comet shape | Oxalic | 2.0 |
| 5 | 10 | 1.80 | - | - | - |

## TABLE LIII

### FRACTIONS OBTAINED FROM BEECH LEACHATE OF AUG. 12, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 8.70 | - | - | - |
| 2 | 10 | 4.10 | 0.64 | - | - |
| 3 | 10 | 1.45 | - | - | - |
| 4 | 10 | 3.90 | Comet shape | Oxalic | 2.0 |
| 5 | 10 | 1.60 | - | - | - |
| 6 | 20 | 1.70 | - | - | - |

## TABLE LIV

### FRACTIONS OBTAINED FROM BIRCH LEACHATE OF AUG. 12, 1948

| Fraction | % Solvent | Ml. of 0.01 N Barium Hydroxide | $R_f$ Value | Acid | Amount (mg.) |
|---|---|---|---|---|---|
| 1 | 10 | 28.30 | - | - | - |
| 2 | 10 | 10.75 | 0.69 | - | - |
| 3 | 10 | 10.15 | - | - | - |
| 4 | 10 | 22.10 | Comet shape | Oxalic | 17.0 |
| 5 | 10 | 8.60 | Comet shape | Oxalic | |
| 6 | 20 | 5.00 | - | - | - |
| 7 | 20 | 3.50 | 0.41 | Malic | 3.0 |
| 8 | 20 | 3.00 | 0.41 | Malic | |
| 9 | 20 | 1.95 | - | - | - |
| 10 | 35 | 4.55 | - | - | - |