Characterization of Leachate from Plant Foliage 1, 2

J. V. Morgan 3 and H. B. Tukey, Jr.
Departments of Botany, and Floriculture and Ornamental Horticulture, Cornell University, Ithaca, New York

For more than 150 years reports have appeared in the literature indicating that a wide diversity of plants can lose mineral nutrients from their leaves through the leaching action of rain, mist, and dew. With the advent of radioisotopes the phenomenon was conclusively demonstrated (4, 5, 13) and in recent years, has been the subject of several reviews (7, 9, 12). Not only are mineral nutrients leached, but also large amounts of organic metabolites are lost from many plants. Chromatographic and electrophoretic analysis of the leachate from bean leaves (Phaseolus vulgaris L.) demonstrated the presence of several amino acids, carbohydrates, and organic acids (11, 12).

Since leaching is such a widespread natural phenomenon (12), it is of interest to characterize more completely the nature of the organic fraction of plant leachates. This paper reports the results of analyses of the leachates from several plant species.

Materials and Methods

The range of plants utilized included bean (Phaseolus vulgaris L. cv. Tendergreen) beet (Beta vulgaris L.), carnation (Dianthus caryophyllus L.), cauliflower (Brassica oleracea var. botrytis L. cv. All Year Round), chrysanthemum (C. morifolium Ramat. cv. Indianapolis White), cucumber (Cucumis sativus L. cv. Ohio Mr. 17), Peperomia obtusifolia A. Dietr., squash (Cucurbita pepo L. cv. 414 Seneca Zucchini Hybrid) and tomato (Lycopersicon esculentum Mill. cv. Fireball).

Seedlings of bean, beet, cauliflower, cucumber, squash, and tomato, and uniformly rooted cuttings of carnation, chrysanthemum, and Peperomia were grown in aerated Hoagland and Arnon’s (3) complete nutrient solution. Nutrient solutions were changed at 10-day intervals.

Plants were leached when they were of a suitable size: after 10 to 20 days in the case of rapidly growing plants such as beans and after 40 to 50 days in the case of slower growing plants such as Peperomia.

The plants were leached by subjecting them to an atomized mist of distilled, deionized water (15 ml/min/plant) for a period of 24 hours. The water after passing over the plants was channelled through exchange resins, from whence it passed to a plastic container to be recirculated by a centrifugal pump. Plants were grown and leached in the greenhouse in natural photo-period at minimum night temperatures of 21° and maximum day temperatures of 32°.

Fractionation and Analysis. The leachates were fractionated on cation-exchange exchange resins, Amberlite IR-120 and IR-45, respectively. The amino acids were eluted from the cation resin with 1 N HCl and the eluate concentrated to 1 ml under vacuum at a water-bath temperature of 50°. The amino acids were separated by 2-dimensional chromatography on Whatman No. 1 paper [modified after Dent et al. (2)] in phenol: water (4:1, adjusted to pH 5.5 with NaOH) and either butanol: acetic acid: water (9:1:2.9) or collidine: lutfidine (1:3, just saturated with water). Spots were located with 1% ninhydrin in 95% ethanol, developed at 80°, and were identified by comparing with known standards and with standard maps prepared by Steward et al. (10).

The organic acids were eluted from the anion resin with 0.3 N HCl and concentrated to a small volume. Separation was carried out by 1-dimensional descending chromatography on Whatman No. 1 and 4 papers. Three solvent systems were used, butanol: acetic acid: water (120:30:50) and ethanol: ammonia: water (160:10:30) as described by Smith (8) and phenol: water: formic acid (75:25:1) as recommended by Block et al. (1). Acidic spots were located on dried chromatograms by spraying papers with 0.04% bromophenol blue in 95% ethanol (6). Some papers were later sprayed with p-dimethylaminobenzaldehyde in acetic anhydride (8) and developed at 90° for a few minutes. This method proved very useful for the identification of many of the Kreb’s cycle acids and acids chemically related to these. Other papers were sprayed with an aniline-xylene reagent (8) and developed at 100° for 10 minutes.

The neutral fraction was again passed over anion and cation resins to remove possible contamination and the eluate was concentrated under vacuum. Separation and identification of the sugars was carried out by 1-dimensional descending chromatography, using butanol: acetic acid: water (9:1:2.9) and

1 Received Oct. 28, 1963.
2 This investigation was supported by United States Atomic Energy Commission Contract AT (30-1)-2598. Taken in part from the M. S. thesis of J. V. M. who held a Kellogg Foundation Fellowship during these investigations.
3 Present address: Department of Horticulture, University College, Glasnevin, Dublin, Ireland.
isopropanol: water (160: 40) as solvent systems. Aqueous isopropanol proved to be the better solvent and good separation was obtained on Whatman No. 4 paper after 18 hours. Papers were sprayed with aniline-diphenylamine (8) and developed at 90° for a few minutes.

All chromatographic work was carried out in an air conditioned room at 21°. Chromatograms of all fractions were run in conjunction with known standards and were examined under ultraviolet light, both before and after development.

Results and Discussion

Cationic Fraction. Analyses of the basic fractions of the leachates from all species revealed the presence of several ninhydrin positive locations corresponding to known amino acids, as shown in table I. The amino acids were present in the leachates in such quantity as to be recognized by not only their relative position on the chromatogram, but also by their color reaction with ninhydrin. In all, 21 amino acids were leached from the foliage of these species. All 7 species lost practically the same array of acids, except for some minor differences. For example in the leachate from bean, the only species previously examined (11, 12), 3 amino acids not previously recorded were identified in these experiments. They were β-alanine, γ-aminobutyric acid, and phenylalanine. However, 3 amino acids, glycine, methionine, and histidine, reported previously, were not detected. The absence of glycine and methionine in the leachate from bean is probably due to their low concentration in this particular sample. The spot for histidine may have been masked by 2 large spots with the characteristics of peptides around its position on the chromatogram.

Tryptophan appeared to be present in the leachate of carnation and cauliflower, but was not detected in other species. Hydroxyproline showed up consistently in carnation leachate only.

Of the amino acids detected, alanine, aspartic acid, γ-aminobutyric, glutamine, glycine, isoleucine, leucine, lysine, and serine were present in the greatest quantities generally. A number of unidentified ninhydrin-reactive spots were also noted in several chromatograms.

An examination of the anion fractions of the leachate revealed spots corresponding to 13 organic acids as summarized in table II. The presence of citric, fumaric, glycolic, lactic, maleic, malic, and malonic acids was previously reported in bean leachate obtained by an immersion leaching technique (12). Maleic acid was not identified in bean leachate in this study, but one additional acid, tartaric, was located.

From table II, it can be seen that organic acids were not leached to the same extent from every plant species. There appeared to be some slight specificity for certain acids, as has been found to be the case with mineral ions (12, 13). In general, citric, fumaric, lactic, malic, and tartaric acids appeared to be more readily leached, as these were found in the leachates of all 7 species. Beet, tomato, Peperomia, and especially chrysanthemum lost considerable amounts of these important metabolic compounds. Aconitric, citric, fumaric, maleic, and succinic also were lost readily. It is possible that all of these acids can be leached from each species and their conspicuous absence from the leachates of some may have been due to their involvement in metabolic reactions at the time.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Plant Species</th>
<th>Bean</th>
<th>Beet</th>
<th>Carnation</th>
<th>Cauliflower</th>
<th>Chrysanthemum</th>
<th>Peperomia</th>
<th>Tomato</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>arginine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>asparagine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>aspartic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-alanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>γ-aminobutyric</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>glutamic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>glutamine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>glycine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>histidine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>hydroxyproline</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lysine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>leucine-isoleucine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>methionine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>proline</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>serine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>threonine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>tryptophan</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>valine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
of leaching. It is also possible that even though they were leached, the concentrations in the leachates were too dilute to be detected by chromatographic techniques.

With all the leachates an acidic spot or streak was found close to the origin on the chromatogram. This appeared to be similar to the acidic glycosides previously reported in bean leachate (11, 12). Some other acidic spots remained unidentified on several chromatograms.

Analyses of the neutral fraction of the leachates were also made. Carbohydrates, in the form of sugars were detected in these samples, including raffinose, sucrose, glucose, and fructose. Tukey and Tukey (12) also noted traces of lactose in squash leachate. The concentrated carbohydrate fractions also contained considerable amounts of unidentified pectinaceous substances, which gave a positive reaction with phloroglucinol.

There was no evidence for the presence of other important metabolic sugars such as xylose, ribose, deoxyribose, and ribulose. This is possibly due to their low concentrations in plant tissues, and also to the fact that they are bound up in forms which are not easily leached.

Discussion

All of the common inorganic nutrients found in plants can be leached, including both the macro- and micronutrients (13). In addition, large quantities of organic materials are also leached, as shown in this paper. These include 21 amino acids, 14 organic acids, 5 simple sugars, and other carbohydrate materials. The list of materials leached from plants now includes 61 metabolites.

The loss of such metabolically important compounds increases the significance and importance of the leaching phenomenon. Leaching is not simply a method of elimination of metabolic waste products as has been suggested (7), but is another factor to consider in the growth and development of plants.

Summary

Plant metabolites may be leached from foliage by the action of rain and mist. Organic materials in the leachates from 7 different species were fractionated by exchange resins and analyzed by paper chromatography techniques. Twenty-one amino acids and amides were detected in the cationic fraction of the leachates. All 7 species lost the same array of amino acids, with minor differences. Fourteen organic acids, including Kreb's cycle acids were detected in the anionic fraction. Leaching of organic acids was more specific for each plant than in the case of amino acids. The neutral fraction contained 4 free sugars, polysaccharides, and other carbohydrate materials.

Literature Cited

of salts in higher plants. Handbuch der Pflanzen-
physiologie IV: 615-37.
10. STeward, F. C., R. M. Zacharius, and J. K. Pol-

Kinetics of Rb Absorption by Excised Barley Roots under Changing Rb Concentrations. II. An Interpretation of Kinetic Data 1

T. Tanada

Mineral Nutrition Lab., SWC, ARS, United States Department of Agriculture, Beltsville, Maryland

In studies of salt absorption by plant roots, the rate of uptake is usually expressed as a simple function of concentration. From data plotted in such a manner, however, only limited interpretation can be made. Judicious use of the Michaelis-Menten equation, which is based on mass action expression of steady state kinetics, makes it possible to analyze still further certain aspects of ion absorption; but, the limitations of this concept must be recognized. Among the first to use it in salt absorption by plant roots were Epstein and Hagen (3) who applied it to elucidate the nature of the inhibition of Rb uptake by other cations. The Michaelis-Menten equation can be employed to give a linear relationship between absorption rate and concentration over a limited concentration range. Over a wide range in concentrations, however, a nonlinear relationship is observed. This has been interpreted as indicating the participation of 2 sites or processes in cation uptake (5).

In this work, it was found that the rate of Rb uptake (\(v\)) by barley and mung bean roots can be expressed as a function of concentration (\(C\)) over a wide range in concentrations, from about \(10^{-6}\) to \(5 \times 10^{-3}\) M, by an equation similar to the Freundlich adsorption isotherm. Thus,

\[
v = kC^n
\]

or

\[
\log v = n \log C + \log k
\]  

where \(n\) and \(k\) are parameters. An expression of this form appears to fit data obtained over a limited range by earlier workers (12, 13).

Equation I has been used in this paper to derive an expression which relates the concentration to time when the concentration is changing during absorption. To do this, equation I is rewritten so that

\[
v = -\frac{dC}{dt} = kC^n
\]  

where \(\frac{dC}{dt}\) is the rate of concentration change or the amount of Rb absorbed during a short interval. Integration of equation Ib in the region of \(C\) gives

\[
C^{(1-n)} = -k't + C_0^{(1-n)} \quad (n \text{ other than 1.0})\]

where \(C_0\) is the concentration at time zero.

Materials and Methods

Barley seeds (Hordeum vulgare L., var. Atlas 46) were germinated and the seedlings grown in darkness with roots in aerated, \(4 \times 10^{-4}\) M CaSO\(_4\) solution at 22°. When the seedlings were 4 days old, they were selected for uniformity in size. Their roots were cut off with a razor blade about 1 cm below the seed. The roots were washed several times with deionized water. Small amounts of roots were quickly blotted with filter paper and weighed. From 0.2 to 1.5 g of roots were placed in 20 to 50 ml of absorption medium, the amount and volume depending upon the Rb concentration. At low Rb concentrations roots were prewashed for about 20 minutes with \(10^{-3}\) M Tris to remove external Ca. Ca inhibited the uptake of Rb at low concentrations. Distilled water redistilled from a glass still was used to make up Rb solutions of low concentrations. To avoid the inhibitory effects of H ions, \(10^{-3}\) M Tris was used as a buffer. The initial pH was 8.3, and it decreased

1 Received Nov. 14, 1963.