THE CHEMICAL ANALYSIS OF PLANT TISSUES*

The work of this committee has been undertaken at the request of the executive board of the Society and in response to numerous expressions of interest from the members. In order that the purpose in view may be understood at the outset a statement of the proposed scope of activity seems to be desirable.

Plant physiologists operating in such applied fields of investigation as agronomy, horticulture and pathology have frequently experienced need for examination of their problems and results in terms of chemical composition of tissues. In many cases it is not feasible to employ a specially trained chemist for this purpose. Moreover, the earlier stages of this phase of the work call generally for the comparative results of somewhat routine chemical analyses. These may point the way to refined qualitative examination and more accurate quantitative results, for which the services of an expert will be required. There is considerable recent evidence to support the practicability of advising competent but less skilled workers in the use of general methods of chemical analysis, where comparative results are concerned. It should be recognized clearly, however, that no such recommendations as are here to be proposed can carry any guarantee of infallibility with all materials and in all situations. A reasonable exercise of critical faculties is invariably demanded of those who would attempt the execution of chemical analyses.

It is not the aim of the committee to attempt to treat completely the applications of chemistry to plant analysis. This is done already in a rather complete manner in such compilations as the Methods of Analysis of the Association of Official Agricultural Chemists. The methods described in this book, however, are not intended for research purposes and, like most encyclopaedic treatises, lack detailed relation to the problems of physiology. Materials of physiological interest are recognized as foundational to this report. For the present, attention will be given chiefly to the much used phases of analysis covering carbohydrates and nitrogenous constituents. In this capacity it is hoped to incorporate current improvements, add discussional approaches to the work and, in general, frame recommendations which may serve as more flexible and specialized directions than are extant in published form. In no sense is dictation of procedure to be presumed upon the experienced investigator. On the other hand, it is suggested that the committee serve as a clearing house

* Recommendations of the Committee on Methods of Chemical Analysis for the American Society of Plant Physiologists.

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of information in which the contributions of experienced workers may be made available for those attempting the preliminary application of chemistry to problems of plant physiology.

A stereotyped method cannot be generally applicable to all the varying complexes of factors in different types of tissue. Perhaps the empirical methods of chemical analysis now generally used will not suffice to delineate accurately the metabolic changes of plants. Nevertheless, there remains for the present an initial span of applied chemistry to which general methods are applicable when modified to meet special requirements. The committee will endeavor to render service in this field, presenting its suggestions from time to time in this Journal. Cooperation is being extended by many workers in plant science whose contributions are herein duly acknowledged. The work will be continued in proportion to its apparent usefulness.

In the present section of the recommendations only general principles of certain phases of the analysis will be presented. Subsequent sections will deal more in detail with special topics.

Sampling

In controlled plant production the variability of composition has not received the attention accorded to yield.

Localization of metabolic effects has been stressed by horticulturists. Where such effects are confined to small tissues it becomes difficult to sample even for micro-quantitative analysis. It is generally feasible, however, to deal separately with the various organs of the plant.

Uniformity of size and appearance of individual plants and organs thereof give no certainty of uniform composition. Sufficient material must be taken to compensate variability in the plant or organ involved. The lower limits of this value must be determined experimentally and might profitably be subjected to cooperative testing. Because of the variability mentioned, as well as the requirements of certain analytical processes, it is advisable to analyze duplicate samples. When composition becomes constant with increasing size of the sample, variability has been compensated.

From twenty to one hundred plants or parts thereof, depending upon the number of uncontrolled variables in production, should suffice for a representative sample. In general, it is advisable to select at least ten times the amount of tissue to be taken as a sample for analysis. So far as possible the minimum of dry matter available from controlled plant cultures should be held at 25 grams.

Extraction

It should be recognized that some enzymatic changes proceed rapidly in crushed plant tissues. Expedition and relatively low temperatures\(^2\) thus become important features of this treatment. The requirements probably vary with the species used and the cultural treatment.

When the extract of the fresh plant tissue is desired, this should be accomplished rapidly in a suitable mill with the addition of liberal amounts of distilled water. Plant fibers will not pass through the sieve plates of a meat chopper, but simple corrugated plates, as in the Nixtamal mill\(^3\), are effective. Qualitative tests upon the extract will determine the extent of washing necessary. The proportion of water used in the grinding process should be adjusted to the degree of succulence of the tissue. Such extracts invariably filter with difficulty, on account of colloidal constituents, but may be washed on a thick pad of paper pulp on a Buchner funnel over a vacuum flask. The filtered extract should be heated to boiling immediately to destroy enzymes, adding a slight excess of calcium carbonate to prevent hydrolysis of sugars when the extracts are appreciably acid. As colloidal material flocculates on standing, immediate analysis is preferable to preservation. Separate aliquots should be acidified with a weak acid (acetic) at the boiling point for recovery of soluble proteins.

When the solubility of colloidal materials, particularly of proteins and dextrins, may be neglected, the most generally convenient and common method of extraction is by hot alcohol. This has the advantage, of course, of immediately checking enzymatic changes of composition. Two procedures are in use. In one, sufficient 95 per cent. ethyl alcohol is used to give at least 80 per cent. concentration thereof as diluted by the water of the tissue. This volume of alcohol is heated to boiling, the cut tissue is dropped in and boiling continued for 15 minutes. Sections of the tissue should be relatively thin to favor prompt penetration of the alcohol, 2 to 3 mm. for succulent plants, and less for woody ones. If allowed to cool slowly material insoluble at room temperature will separate and may be strained off. Several washings with 80 per cent. hot alcohol treated in this manner should complete the extraction, the solutes being recovered by evaporation of the extract at low temperature. If aliquots are taken from the alcoholic extract it should be sampled at uniform temperature, as it has a considerable coefficient of expansion.

Another method includes 50 per cent. alcohol as the effective extracting agent. This has the advantage of high solvent power and in some cases has been found as effective as water. The hot extract is preserved

\(^3\) Enterprise Mfg. Co., Philadelphia.
by increasing the alcohol concentration to about 80 per cent. Colloidal or other material which separates on cooling should be recovered with the insoluble residue. Large Buchner funnels serve conveniently for the percolation and extraction of considerable masses of tissue.

As a general value it may be accepted that somewhat less than 2 liters of water accomplishes practically complete extraction of succulent tissues. The action of hot alcohol seems to be complete at smaller volumes. It has been found that 100 grams of apple spur tissue is exhausted by about 1250 cc. of 80 per cent. alcohol. The alcoholic treatment denaturizes most proteins and renders them insoluble, but all methods of desiccation also seriously reduce the amount of soluble proteins.

The use of calcium carbonate is usually recommended in connection with extraction, chiefly to prevent the inversion of sucrose by organic acids while heating. Some workers have employed ammonia for this purpose, and it should be more effective in penetrating intact cells. This matter deserves experimental attention. In any event, it should be recognized that such additions almost invariably alter the reaction of the plant sap and are liable to disturb the natural distribution of solutes. For the latter reason also, alkalinity of the extract is to be avoided over appreciable time periods. However, an excess of solid calcium carbonate is not objectionable in this sense. To minimize alterations during the recovery of extracts from distinctly acid tissues, it is advisable to conduct a parallel extraction without the use of a neutralizing reagent.

Plant extracts should be analyzed as promptly as possible. Unavoidable delay is more permissible with alcoholic extracts than watery ones, due to the flocculation of colloids in the latter case, as well as the necessary use of preservatives. Burrell, of Ohio State University, found values for amino and nitrate nitrogen constant in alcoholic extracts for two weeks. Exposure to light should be avoided. The insoluble residue should be oven-dried and ground as fine as feasible for further analysis, preferably to 100 mesh. For this purpose the Dreef pestle mill is unusually satisfactory.

**Desiccation**

Past investigation of chemical composition in plant tissues have suffered rather generally from failure to consider the effects of the method of desiccation employed. This is especially true of "air drying," where enzymatic changes may become extensive and mask the real composition of the tissues. Thomas, of Pennsylvania State College, recommends a temperature of 60° C. in vacuo as maintaining with least disturbance the

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4 Formerly stocked by Eimer and Amend, New York.
original composition of the tissue. Spoehr and Chibnall advise that different types of plant tissue are widely variable in their requirements of desiccating treatment. Link has discussed the factors involved in heat drying. It is not possible to avoid considerable denaturing of proteins by heat drying, and in highly acid tissues sucrose suffers inversion. In general, with masses of tissue too great for treatment in vacuo, drying at 60°C with forced ventilation minimizes hydrolytic and respiratory changes without caramelizing the sugars. Many types of tissue permit also a preliminary heating at about 100°C for a matter of 30 minutes to kill the tissue. Other types, rich in colloidal materials, are more resistant to desiccation and require preliminary alcoholic extraction.

Lipoid removal

The necessity of clearing cell walls from fatty and waxy components in order to facilitate extraction and the entrance of watery solvents is rather obvious and well known. To a considerable extent this function is accomplished by the hot alcoholic extraction previously mentioned. Few other lipoid solvents are applicable to fresh, watery tissue and so universal in solvent effect. Sando, of the U. S. Bureau of Plant Industry, considers alcohol the most efficient solvent for lipoids in the sense that the latter term includes fats, fatty acids, waxes, sterols and phospholipoids. Certainly this extract is of heterogeneous character and has little significance for expression in quantitative terms. It may be further fractionated by extracting the recovered solids with ether.

When dried tissue is to be dealt with, ordinary (ethyl) ether is to be preferred for extraction. The low boiling point and general handling qualities of this solvent have given it general favor. It must be anhydrous and free from alcohol, or sugars and other non-lipoidal constituents will be removed. This form is advertised by manufacturers at a reasonable increase over the cost of commercial ether. It should be possible to obtain it true to specifications. According to Sando, the lipoidal extraction of dry material is incomplete as compared with the use of alcohol on fresh tissues. The use of ether should leave all carbohydrates and non-lipoidal nitrogenous constituents in the tissue.

Determination of moisture

Regardless of the completeness of removal of water from tissue, as usually stored and handled it acquires the "air dry" state by attaining moisture equilibrium with the contiguous atmosphere. Hence the neces-

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sity of determining the actual dry weight used for analysis. It is probably sufficient, as a general principle, to employ the same temperature and desiccator service throughout a single investigation or related ones. Where drying is accomplished in vacuo a standard value of barometric pressure should be selected for the temperature employed, as the percentage of water remaining in the tissues varies appreciably with moderate differences in pressure. In the relatively rare cases where volatile organic compounds are present in appreciable proportions their recovery in the process of drying the fresh tissue presents, of course, a special requirement.

Expression of results

The common practice of stating composition as percentages of the dry matter may fail to denote changes in the absolute amounts of constituents. In this respect the amount of constituent per plant, or multiple thereof, is advantageous. CHIBNALL stresses the possibility that variations of less abundant constituents may be masked by increases of inert wall material. In this event the basis of fresh weight will have particular value in the statement of results, unless the water content is rather constant. Perhaps the basis of dry matter plus bound water content, if generally practicable of determination, will be found more significant. Physiologists are attempting to determine what bases for expressing results will best represent the concentration of metabolic materials in the living tissues under variable conditions. In view of its long-standing usage it seems advisable to retain the expression of percentages in the dry matter, employing also the basis of green weight and such others as appear particularly significant.

This report was organized by W. E. TOTTINGHAM for the Committee.

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