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A SURVEY OF THE USE OF CHEMICAL TISSUE TESTS FOR DETERMINING THE MINERAL STATUS OF CROP PLANTS

by

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Saussure in 1804 made the first approximate analysis of leaves and seeds of a variety of crop plants for mineral elements and showed that the soil had a profound effect on the amount and composition of nutrients in the ash [98]. Since then ash analysis has been extensively used in interpreting fertilizer experiments and for determining deficiencies and toxicities of mineral elements in crop plants [1, 2, 3, 49, 64, 73, 81, 83, 97, 104, 105, 108]. These methods are often elaborate and time-consuming. It is not surprising therefore that attempts have been made to speed them up. This has been done in two ways. Firstly by the use of the spectrograph, polarograph, spectrophotometers and absorptiometers to facilitate quicker and often more exact determinations of the ash constituents, and secondly by the use of rapid chemical tests on fresh plant tissues. This review is concerned with the latter procedure.

Definition

Tissue tests involved the use of chemical methods for determining certain fractions of the total mineral nutrients in fresh plant material as distinct from ash-analysis procedures for total amounts in dry matter.

Tissue tests are often designed for use in the field to supplement information from crop symptoms and simple soil tests. Under these conditions they must be made with the minimum of apparatus and time. The chemical tests must be simple yet capable of giving semi-quantitative results that are reproducible to 10 per cent. The accuracy is below that regarded as essential for ash analysis, nevertheless the fact that a number of tissue tests can be made quickly on the same sample tends to outweigh the disadvantage.

Field Sampling Methods

The problems associated with sampling plant material for chemical analysis have been recognized from early studies and are common to tissue tests and ash analysis. It is often impossible to determine the mineral elements in whole plants, and this led early investigators, in particular Heinrich [56], Atterberg [2] and Alway [1], to confine sampling to particular tissues. The choice of plant parts has been subject to debate and conclusions are often based on little experimental work. The leaf has been regarded as a suitable sampling unit and when actively metabolizing it is likely to reflect nutrient deficiencies or excesses sooner than other plant portions. Three types of leaf sampling have been advocated, (*a*) taking morphological samples comparable in age and position [64, 66, 67, 80-86, 96, 97, 104, 105, 108, 109], (*b*) taking all leaves from a certain number of plants in a population [11, 73], (*c*) making a random selection of leaves from a number of plants [110]. Gossard [50] showed that the error in random sampling of leaves was greater than in those taken at a fixed morphological position. At Long Ashton the approach to the sampling problem has been physiological, based on visual and chemical data on the mobility of mineral nutrients within the plants [80-86, 92]. K, Mg and P deficiencies usually appear in older leaves and these nutrients are readily translocated to younger leaves, whereas Ca, B and Fe deficiencies appear in young leaves and are less mobile. Thus K, Mg and P should be determined in older leaves, and Ca and B in younger leaves. It is clear that time involved in differential sampling would not be worthwhile, so attempts have been made to get information from a composite sample for a range of nutrients [49, 80, 81, 82, 83, 102, 104, 108]. At Long Ashton a study was made of the nutrient gradients in crop plants. It was shown that the mid-stem leaves, in most crops, reflected nutrient changes in the whole plant satisfactorily and indicated the effects of fertilizer treatment. This physiological position also gave continuity of sampling throughout the season [80-86, 92].

PREPARATION OF TEST SAMPLES

The entire leaf lamina has been used by a number of workers for ash analysis [49], others including Hance [52, 53, 54], Yuen [114], Borden [5] and Lagatu and Maume [64] have used punched portions of it for test. Emmert [27-42] used the leaf margins, whereas Gauch and Wadleigh [48] used root tissue for analysis. For chemical tissue tests, however, preference has been given to petiole or stem portions. The assumption is that more 'unassimilated' nutrients are present in petioles than in the leaf lamina. Thornton [107] determined the P content of translocation or storage tissues rather than leaf laminae. Emmert [27-42] sampled petioles of lower leaves of tomato, as their growth rate is less than in the young leaves. At Long Ashton petioles or midrib portions are sampled for field tests whereas leaf laminae are used in maceration procedures in the laboratory. Experiments showed that the extracts of nutrients from petioles reflected the mineral status of the leaf as well as or even better than leaf extracts. Petiole extracts were often free from pigment, and tests could be made directly without clarification whereas leaf extracts had to be clarified with Darco G60 carbon before testing [82]. It was more expedient therefore to adopt petiole analysis for field use. Leaf-lamina extracts prepared in maceraters may be used for laboratory tests [82, 86]. It should be stressed that fresh material must be tested as soon as possible after sampling to avoid moisture losses.

EXTRACTION METHODS

The first attempts to determine mineral nutrients in fresh plant material were made by Hoffer and Frost [60] and Hoffer [59] who showed by a qualitative staining technique that Fe accumulated in the nodal tissues of maize when K was deficient. Other workers, however, did not find the test of value, and Kruger [63] and Neeb [79] showed that the results did not apply to oats and sugar beet, respectively. MacGillvray *et al.* [74] determined K in sections of tomato petioles immersed in platinic chloride by the relative abundance of potassium platinic chloride crystals.

Expressed Sap

A later development was the analysis of expressed sap for nitrate-N, P and K. This extraction was done by grinding fresh plant material and straining through a muslin or silk mesh. McCool and Weldon [77] extracted sap under pressure of 1 ton per sq. inch and determined P and K in the extract, Fonder [43, 44] tested for Ca and Mg in the sap of beans, and Cook [23] for nitrate-N in leaf extracts of cereals. Pettinger and Thornton [107] showed that the NPK content of maize was closely related to manurial treatment, and Craig and Halais [24] found that the P content of sugar-cane juice reflected P treatment and yields of sugar better than soil analysis. Carolus [14, 16, 17, 18] working with potato showed that limiting values could be fixed for N, P, K, Mg and Ca in the extracts, but Poehlman [93] working with soya bean failed to determine standards for nutrient deficiencies. McCool [76, 77] showed that the composition of the expressed sap was affected by variations in pressure and by differences in attaining the final pressure. Gassner and Goez [47] improved the reproducibility of the results by killing tissues first before pressing. Eaton [26] found that B in the sap of plants varied little with B supply, and Fudge [45] showed that the B content of expressed sap was low despite an increase of total B, but at toxic levels the concentrations in the sap increased more markedly than total B. Smith [100] found some correlation between B content of plant sap and the application of borax.

Plant exudates either from natural secretion via hydathodes or from cut ends of plants have been examined for mineral nutrients. Lowry and Tabor [71] and Lowry *et al.* [72] collected sap from cut ends of maize stems and showed that K and P content reflected the treatment, but the N level did not.

SOLUTION METHODS

Before discussing the types of extractants used, it is as well to mention briefly the methods employed in extracting mineral nutrients. These fall into two groups (*a*) diffusion methods for the extraction of the easily soluble nutrients [3, 4, 8, 12, 18, 22, 25, 27, 80, 81, 83, 85, 92], and (*b*) maceration methods using the Waring blendor [61, 82, 86, 112, 113], Folley macerater and pyrex-glass micro-mills [84, 86]. The diffusion method is a field procedure whereas the

maceraters are used in the laboratory for semi-quantitative work. The pyrex-glass maceraters are used for extracting mineral micronutrients [84, 86].

Water.—Workers have used a variety of solvents to extract mineral nutrients from fresh plant material. Thus Nightingale [87, 88] used cold water for extracting Ca from sugar cane as did Marsh [75] from tobacco, bean, maize and oats. Burkhart and Collins [9], Burkhart and Page [10] and Linberry *et al.* [66, 67] used boiling water to extract N, P, K, Mg and Ca from peanut, cotton and strawberry and showed that there was a tolerably close relationship with fertilizer application and that P in particular was in agreement with the total amount present. Brown [8] and Brickley [7] determined nitrate-N and P in water extracts of sugar-beet and potato leaves respectively. Scarseth [99] showed that drying plant material altered the proportion of K soluble in water. Nicholas [84-86] has recently used glass-distilled water to extract Fe, Cu, Zn, Pb, Mn, Co and Ni from tomato leaves in special glass maceraters.

Organic Acids.—Emmert [27-42] was among the first to use 2 per cent acetic acid for the extraction of nitrate-N, P and K from vegetable crops, and Carolus [12-21] found that extracts of stems and petioles reflected the nutrient content of the whole plant. The extraction method has been used by a number of workers, including Hill and Johnston [58] for determining Mg deficiency in apple, Cook [22] for K deficiency in legumes, Lorenz and Minges [70] for the NPK status of lettuce, and Ulrich [49] for five major nutrients in clover petioles. Lorenz [69] found that changes in the acetic-acid extract of potato petioles were more marked than the total nutrient content. Thorne and Wallace [106] showed that the acetic-acid extracts of green leaves contained more iron than those of chlorotic leaves. At Long Ashton acetic acid has been employed as an extractant for a range of crop plants using macerater methods [82, 86].

Buffered Solutions.—In 1935 Morgan introduced the now famous acetic acid-sodium acetate solution (pH 4.8) for the extraction of mineral nutrients from soils [78]. He suggested that the extractant should be useful for preparing plant extracts. At Long Ashton Morgan's reagent has been extensively used to extract mineral nutrients from predetermined portions of plants either by the diffusion process or by disintegrating in a Waring blendor or a macerater [82-86]. Walsh [111] and Harrington [55] used the reagent for a range of crop plants, Davidson and Blake [25] for peach, Potter and Percival [94] for apple petioles. Hester [57] and Wolf [112, 113] and Hunter [61] used a Waring blendor to extract nutrients with the acetate buffer. Peech and English [90] have described chemical tests for mineral nutrients in Morgan's extract of soils and they suggest that the same extractant could be used for plants.

Hydrochloric Acid.—This acid has been frequently used to extract nutrients from dry matter. Thus Lundegårdh [73] used n.HCl extracts of plants for spectrographic analysis, and Oserkowsky [89], Jacobson [62], Thorne and Wallace [106], Somers and Schive [101] and Liebich [65] used different concentrations of HCl to extract 'active' iron from plant tissues. Greenhill [51] compared acetate buffer extracts of fresh plants with those of dilute HCl and found that the P status was better shown in acid extracts. The acid extraction took 2 hours to reach completion. At Long Ashton [85, 92] redistilled HCl (constant-boiling mixture) has been used to extract heavy metals present in excess in fresh plant tissues.

Sulphuric Acid.—Emmert [30] used the dilute acid to extract phosphate from tomato and lettuce leaves.

Ethyl Alcohol.—Beauchamp [3, 4] extracted 'crude chlorophyll' with alcohol and determined N, P, K, Mg and Ca in the extract. The residues left after extraction were also analysed. The K in the extract correlated well with other diagnostic methods when plants were grown in low-K soils. The K in the leaf residue however showed no relation to treatment.

Other extraction solutions include reagents used for determining the mineral nutrients. In the Purdue tissue tests [99, 107] sodium cobaltinitrite and alcohol are used to extract

K; ammonium molybdate and dilute sulphuric acid to extract P, and diphenylamine and sulphuric acid to extract nitrate-N.

Chemical Tests

Most of the chemical methods described in early papers are qualitative, based on microchemical staining techniques or testing extracts with the tissue *in situ* so that nutrients continue to diffuse into solution [99, 107]. Attempts have been made to make the chemical methods quantitative and reproducible. For field work, the chemical tests must be simple in operation and preferably specific for the test nutrient so that there is no need for elaborate chemical separations or heat treatment before testing. Tissue extracts usually contain small fractions of the total nutrients so that interferences of one nutrient with another are less than when total amounts are determined. The Long Ashton methods are designed to satisfy the simplicity of field requirements and in addition to retain a reasonable degree of accuracy and reproducibility [80-83, 85, 92]. In the laboratory the tests used for the macerater extracts are a little more elaborate than field tests, in order to give more precise results [82, 84, 86].

Interpretation of Results

Qualitative procedures are used to determine whether the plants are deficient or not in a particular element. This procedure, although useful for the diagnosis of obvious cases of malnutrition, is of little value for doubtful instances. Semiquantitative methods, however, are better, as standards may be fixed for various elements which correspond with levels of nutrition from normal to deficiency or toxicity of the elements.

Seasonal Trends

At Long Ashton the seasonal trends for the various nutrients have been determined in a variety of crop plants [81, 82, 83]. The results show that K, Mg and in particular nitrate-N fall with season so that differences between normal plants and those deficient in one of these nutrients may be much reduced late in the season. Nitrate-N falls to such low values from mid to late season, irrespective of treatment, that it cannot be used at this stage for the diagnosis of N deficiency. A knowledge of the seasonal trends for the nutrients in crop plants grown at various nutritional levels facilitates the diagnosis of an impending deficiency prior to the appearance of visual signs. The difficulty that some workers have experienced in fixing nutrient standards may in part be due to determining nutrients, once only, during the season without reference to the seasonal changes. Harrington [55] and Nicholas [81, 83] showed that seasonal tissue tests are most important for the diagnosis of mineral deficiencies.

Nutrient Standards

It is clear from tissue-test studies that nutrient standards vary with the type of crop plant and that a detailed knowledge of crops grown in a variety of soils is required before standards can be fixed. A detailed survey of a number of crops has already been made using the diffusion and macerater methods [80-86]. For diagnostic work in the field, differences in nutrient levels of good and poor specimens may be sufficiently striking to facilitate a diagnosis. On the other hand the differences may be small as the so called 'good' specimens may be near the deficiency level and there are no visual symptoms to guide the sampler. In this connection a knowledge of nutrient standards associated with a really healthy plant is essential for a correct diagnosis.

Tissue Tests and Visual Symptoms in Crops

The relation between tissue tests and visual symptoms [109] of malnutrition have been determined in crop plants in long-term manurial trials. Threshold levels defined as tissue-test values coinciding with the appearance of visual symptoms of a deficiency or toxicity have been fixed in a number of crops. The correlation between tissue-test values and symptoms has been close in a number of tissue-test investigations. Discrepancies have been reported however, in particular instances, e.g. in stunted plants where the concentration of the deficient nutrient may be greater per unit weight of tissue than in a larger and more vigorous plant [55, 86].

Tissue Tests in Relation to Crop Yields

As mentioned previously tissue tests have

been related to fertilizer treatment, and the correlation found has often been close. Moreover, attempts have been made to link the values to final yields of crops. This is more difficult, as factors other than nutrient supply have a profound effect on final yields of crop. Nevertheless Craig and Halais [24] and Borden [5] showed that the P content of sugar-cane sap followed the yield curve closely. Nicholas [80-86] has also found that the diffusion and Waring-blendor results for K in barley grown in a number of factorial experiments were closely correlated with yield figures. Moreover, it was possible to suggest values in the tissue extracts, determined at intervals during the season, over and above which a further increase of grain yield would be unlikely. Further experiments are also in progress with Mn in relation to final yields [86].

Tissue Tests in Relation to Soil Analysis

Little information is available on the relation of tissue tests to soil analysis despite the fact that Morgan developed a quick method for examining soils for mineral nutrients [78]. These, however, were qualitative and were modified later by Tinsley and Pizer [103] as semiquantitative methods for determining K, P and Ca in British soils. Thornton *et al.* [107] found that the value of the quick soil tests was enhanced by tissue tests. A number of workers have found tissue tests of greater value than soil tests for diagnosis of a mineral deficiency, as it is difficult to find a chemical extractant for soils that will reproduce the extracting properties of the roots of higher plants. It is known that plants behave differently with regard to the absorption of mineral nutrients from the same soil [49, 73, 81, 109]. Jones and Russell [95] made a tissue-test survey of crops grown on farms in south-west England. Soil analysis was done in 1946 and tissue tests on the crops the following spring. The diffusion method was used and the results were assessed into low, medium and high categories. The correlation between soil and tissue tests, although positive for K and P, was not close.

Tissue Tests and Ash Analysis of Plants

It is not often that comparisons have been made between tissue-test data and those of ash analysis. Such comparisons are of great importance, as ash analysis is an accepted method for the diagnosis of the mineral status of plants, other than for iron [83]. Total iron bears little relation to iron-deficiency symptoms, so that Oserkowsky [89], Lindner and Harley [68], Nicholas [84] and Thorne and Wallace [106] have attempted to fractionate iron in plants.

Burkhart and Page [9] showed that the P content of water extracts of peanut and cotton was closely related to the total content present. Carolus [16, 17, 18] showed that changes in N, P, K, Mg and Ca levels in acetic-acid extracts were similar to those for the total amounts present in the ash. Ulrich [49], on the other hand, showed that acetic-acid extract was a better index of the NPK status than total amounts, and Lorenz [69] found the same in similar extracts of potato petioles. At Long Ashton comparisons have been made between the results of tissue-tests and of ash analysis, at intervals during the season, for crops grown in long-term manurial trials [81, 82, 83]. A close correlation was found for K, Mg, Ca, P and Mn in tissue extracts and the totals present in the ash over the diagnostic range from low to high levels. At luxury levels, however, there was little or no correlation between the two methods. This may be due to (*a*) the 15-minute interval for extraction (diffusion method) being too short for the extraction of larger quantities of the total amounts, (*b*) the immobilization of large proportions of nutrients in the tissues at luxury levels so that they are not extracted by the solution used. Nevertheless over the diagnostic range the correlation is close so that tissue tests can be used instead of ash analysis for diagnosis of malnutrition in crops.

Interaction of Mineral Nutrients

Several interrelationships of mineral nutrients are known. Thus Hoffer and Trost [59] showed that iron accumulated in nodal tissues of maize when K was deficient. Burkhart and Page [9] showed that high K depressed Ca and Mg in water extracts of peanut and cotton.

Conclusions

The apparent simplicity of chemical tissue tests is often misleading. The techniques described including sampling of *fresh* plant material, preparation of test samples, extraction, chemical testing and interpretation

must be done thoroughly, observing all the necessary precautions at each stage. Only when this has been done can tissue tests be of value as a complementary method to other procedures in the diagnosis of the mineral status of crop plants.

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