

A review of plant analysis in Australia

P. D. Handson and B. C. Shelley

State Chemistry Laboratory, Melbourne, Vic. 3002, Australia.

Summary. This review of plant analysis in Australia examines sample preparation, instrumentation, problem analytes, calibration, detection limits, and quality assurance. The issue of turnaround time v. analytical accuracy is discussed and the role of 'plant sap quick tests' in nutrient analysis is assessed. Results of a survey of Australian plant-testing laboratories are included.

Introduction

This review addresses one aspect of a plant tissue testing program, namely, the provision of a timely, efficient, and reliable laboratory service. Information presented in this review was obtained from questionnaires sent to all Australian Soil and Plant Analysis Council (ASPAC) member laboratories involved in plant analysis. Twenty-one questionnaires were returned, and these responses, plus interviews with selected analysts, provide a broad overview of plant analysis in Australia.

Plant samples may be divided into diagnostic and experimental samples. Laboratories tend to treat each category differently. Experimental samples are usually less urgent and are used to help spread the workload. Turnaround times of 3–4 months are not uncommon. Analysis of research samples often requires a higher degree of precision than diagnostic samples, and laboratories may use more precise or more sensitive analytical methods, or more stringent quality control procedures, to ensure the researcher receives the quality of result required. In contrast, results from diagnostic samples are required as soon as possible and these samples receive top priority. A turnaround time of 5 working days is the maximum delay acceptable to most growers and agronomists. Diagnostic samples often only require screening tests, and laboratories may use analytical methods that are simpler and faster, although less precise, than the methods used for the analysis of research samples.

Traditionally, diagnostic samples were submitted to help diagnose suspected nutrient deficiencies, and the results were used to correct the problem in subsequent crops. However, if the test results are obtained quickly enough it is possible to correct deficiencies in the current crop. Increasingly, growers are using plant analysis to ensure proper nutrition during the growth cycle of the current crop. This is particularly true with horticulture and hydroponics but is also becoming evident in broadacre farming.

This trend to crop monitoring means that laboratories are under increasing pressure to produce results quickly. Typically, the cycle of sample collection, shipping to the laboratory, analysis, interpretation, and return of results to the grower must be held to 4–5 working days (I. Grant, Pivot, pers. comm.). Some growers, particularly those with tight irrigation schedules, are requesting next-day service. Laboratories servicing these growers are responding by providing 24 or 48 h turnaround times, often using quick tests on fresh material or sap.

It is a major challenge to all laboratories to strike a balance between analytical accuracy and precision and the provision of a timely service that meets client requirements. Laboratories must balance the use of sophisticated techniques such as inductively coupled plasma–atomic emission spectroscopy (ICP–AES) or X-ray fluorescence (XRF) spectrophotometry with that of simple methodology such as ion-selective electrodes (ISE) or paper test strips. Failure to achieve this balance will see plant testing becoming increasingly irrelevant to grower needs.

Many laboratory procedures of different approach have been developed for plant analysis. For example, tests for elemental analysis range from exotic neutron magnetic resonance (NMR) techniques (currently limited to research applications) to field quick-test kits. These methods usually require destructive sampling, either dry ashing or digesting the sample in 1 acid or a combination of acids. For small sample sets, some laboratories may employ microwave digestion in acids, but most laboratories will digest samples using a controlled temperature heating apparatus.

A common misconception amongst agronomists and growers is that 2 laboratories should report the same result on split samples. In fact, the analytical method used by a laboratory can greatly affect the interpretation of the results and often clouds such expected agreement. The laboratory result is, however, 1 step to obtaining accurate interpretations and recommendations. It is the

accuracy of the recommendation and subsequent positive crop response that is of value to the grower not the absolute result from the test. This fact should be explained to clients to avoid problems arising from comparing analytical results from different laboratories.

Plant analysis in Australia

Timeliness of sample measurements

On average, the laboratories surveyed analysed about 7000 samples/year (range 200–45 000). Thirty-five per cent of these samples are diagnostic (range 0–100%), and the average sample turnaround time (TAT) is 7.5 days (range 3–21 days). Nine of the 21 respondents achieved TATs of 3–5 days, while the remaining 12 reported TATs of 7–21 days. If this latter group is to meet client expectations, their TATs must be reduced. While TAT for experimental samples is less relevant, they do comprise 65% of all samples and it is worthwhile comparing performance in this area. The average TAT for experimental samples was 40 days, with 1 laboratory (>5000 experimental samples/year) reporting a TAT of 5 working days, while others reported TATs of up to 4 months.

The survey responses indicated the following.

(i) Sample number has minimal effect on TAT. Laboratories analysing <5000 samples/year had an average TAT of 7.4 days (range 3–15 days), while for laboratories analysing >5000 samples/year the value was 8.3 days (range 3–21 days).

(ii) Access to high throughput XRF and/or ICP instruments does not necessarily ensure fast turnaround times. The average TAT for laboratories with ICP and/or XRF was 9.5 days (range 3–21 days), more than double the 4-day average (range 3–7 days) for laboratories without this technology. Sample numbers alone do not explain this difference: 2 of the larger laboratories (14 000–15 000 samples/year) reported TATs of 8 days with ICP and 4 days without, while for 2 smaller laboratories (1500–2000 samples/year) the corresponding values were 9 and 3 days. The laboratories compared above offer a similar range of plant analyses including sulfur (S), boron (B), and molybdenum (Mo).

(iii) Laboratories that dried samples for 16–24 h achieved an average TAT of 4 days (range 3–6), while those drying for >24 h (48–72 h) had an average TAT of 11 days (range 5–21). Drying time alone cannot account for this large difference in performance. Other contributing factors include the number of staff and instruments involved in the analysis and data reporting system.

Preparation of samples

Sample washing. Although it is generally not necessary to wash samples for the determination of the major elements, washing is recommended for trace

elements, particularly iron (Fe), aluminium (Al), and zinc (Zn) (Sonneveld and Van Dijk 1982). Rinsing for 10–15 s in demineralised water or 0.1% Teepol or 0.1 mol acid/L followed by a demineralised water rinse is the most common washing procedure. Fifty per cent of the surveyed laboratories washed samples on a regular basis. In most cases a single rinse with deionised water was the preferred method.

Sample drying. There were at least 14 combinations of temperature and time used by the 21 laboratories to dry plant samples. Procedures used included 60°C for 24, 48 or 72 h, 65°C for 24 or 48 h, 70°C for 24 or 48 h, 80°C for 16–48 h, and 105°C for 16–24 h. If variation between laboratories is to be minimised then 1 standard procedure for sample drying, or perhaps 2, should be adopted. Recent discussions with analysts suggest that 80°C for 16–18 h would be a good compromise between a requirement for rapid drying to achieve quick throughput and a reduced risk of nutrient loss at lower temperatures [i.e. nitrogen (N)].

Sample grinding. There was considerable variation among the 21 laboratories regarding desired particle size. The specified fineness ranged from <100 to 2000 µm, and some laboratories did not even have a standard. Particle size can affect precision and accuracy of many analyses, particularly where small sample weights are used (e.g. micro-Kjeldahl or Leco N analysis, or in XRF analysis). Two laboratories that reported Kjeldahl N as a problem analysis because of lack of precision had no particle size standard for their grinding operation.

As grinding is machine-dependent, there is less scope for setting a standard degree of fineness. However, recommended particle size standards for the guidance of members need to be established.

Sample digestion. With the exception of XRF, N by combustion methods, and some aqueous extractions, the bulk of analysis of dried plant material requires a process to destroy organic matter. Most laboratories use acid digestion with commercial or custom-built heating blocks. Ten of the surveyed laboratories used ashing in muffle furnaces either for the full suite of analyses (2 laboratories) or for specific nutrients such as Mo or B, while 19 of the respondents used wet digestion for the bulk of analyses. Nitric–perchloric, nitric–sulfuric, and nitric–perchloric–sulfuric acid mixtures were most frequently used. In some cases hydrogen peroxide was also used to aid oxidation.

The availability of the ICP in many laboratories has seen a change in digestion procedures. A much simpler nitric acid digest can be used to obtain dissolution of the sample, the high temperature of the plasma torch being sufficient to break most molecular bonds, to free component atoms, and to excite their characteristic emissions. The 12 laboratories using ICP were evenly divided between nitric acid digests and nitric–perchloric

acid digests. Reports from analysts using nitric acid to digest samples indicate that the accuracy and precision are as good as with the double-acid mixture and the digestion time is shorter. With the added dangers and higher costs associated with nitric-perchloric acids, the nitric acid digest should be the dissolution method of choice for plant analysis by ICP.

Microwave digestion is becoming more common in plant testing laboratories. Four (20%) of the surveyed laboratories, all with ICP, reported using microwave digestion for some or all analyses. Conversion and use of domestic microwave ovens is not recommended for safety reasons, and there are a number of laboratory microwave oven digestion systems on the market today. Unfortunately, these systems are expensive, starting at around \$A25 000. The technique has many advantages. For example, using high pressure systems such as the Milestone (11 MPa), plant tissue (cellulose, oilseeds, high sugar samples) is digested in about 10 min. Lower pressure systems (0.8 MPa) can take up to 50 min. Most systems accommodate 6–12 samples. The addition of a cooling system and use of multiple carousels of digestion vessels can provide throughputs of 24–50 samples/h. Because the system generates far less acid fumes, large scale acid fume scrubbing systems are not needed and there is less reliance on operator skill for successful dissolution of difficult samples. The choice of microwave system will depend on the type of samples being analysed and the required throughput. This technique is well suited to matrices that are difficult to digest by traditional block techniques or where low detection limits are required.

Instrumentation used in plant analysis

The soil and plant analyst now has a wide variety of analytical tools to choose from, compared with just a few years ago. Analysts are faced with many new options and must decide which technology or which brand of new instrument is appropriate for their laboratory. Acquiring such new technology can be very expensive and analysts must weigh the alternatives and critically assess each new instrument. Analysts must do a thorough cost-benefit analysis in order to establish the equipment necessary to match the analytical requirements of precision, sensitivity, and cost.

X-ray fluorescence spectroscopy

X-ray fluorescence spectroscopy has been routinely used for plant analysis since the early 1970s. Instruments available at that time such as the Phillips PW 1410 could be interfaced with computers for data processing but samples had to be introduced manually. Modern instruments such as the Phillips PW 1404 come with autosamplers holding up to 300 samples, although with analysis times of about 20 min/sample, a table of 72 samples is adequate for a 24 h run.

The main advantage of XRF analysis is that sample preparation consists of drying, fine grinding, and pressing. Detection limits are more than adequate for most elements, ranging from 100 µg/g for major constituents to 1 µg/g for trace elements and heavy metals. Samples as small as 1 g can be analysed using small diameter pressing dies. Low sample throughput (3 samples/h) is compensated by the ability to run 24 h unattended with no safety problems. XRF calibration is matrix-dependent and is based on secondary calibration using material analysed by (primary) calibration techniques such as AAS-ICP.

The technique is subject to spectral interferences that must be corrected on a matrix-by-matrix basis. Sample particle size and mass have profound effects on detection limits and accuracy and uniform fine grinding is essential.

The major impediment to greater use of XRF for plant analysis is the initial capital cost (about \$300 000). Laboratories possessing both XRF and ICP-AAS report that XRF is the method of choice, mainly on the basis of analysis cost. High initial cost limits the use of XRF and only 3 of the 21 laboratories surveyed (14%) routinely used XRF for the determination of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), Zn, manganese (Mn), Fe, Al, silicon (Si), S, phosphorus (P), and chloride (Cl⁻) in plant samples. One laboratory included B in the suite of elements analysed, while another offered the determination of bromine (Br), rubidium (Rb), strontium (Sr), Mo, ruthenium (Ru), and chromium (Cr).

Inductively coupled plasma-atomic emission spectroscopy

The cost of an ICP-AES (ICP) spectrometer is about \$110 000 which is about twice the price of a quality flame atomic absorption spectrometer (AAS). This places it within the financial reach of many laboratories and offers the advantage of being able to determine P, S, and B, in addition to the elements traditionally analysed by AAS. For runs where there are <4 elements/sample, a modern, computer-controlled multi-element flame AAS equipped with an autosampler, such as the Varian SpectraAA 400 or GBC906, has a greater throughput than sequential ICP. For more elements per sample, sequential ICP becomes the more efficient technique.

The cost of operating ICP is only marginally greater than flame AAS, and because no fuel gases are used it can be left running overnight. Detection limits are between 1 and 0.5 times that obtained by flame AAS. The usefulness of ICP for determining heavy elements such as cobalt (Co) and Mo in plant material is restricted by the detection limits.

Spectral interferences are the major source of error and particular care must be taken when analysing unusual matrices or contaminated samples. This problem is more

serious with simultaneous spectrometers, where choice of lines is limited to those selected at the time of instrument manufacture. If interferences are present, spectral corrections are easily made and the higher throughput of the simultaneous instrument (20–30 solutions/h) provides a distinct advantage over a sequential machine (5–10 solutions/h).

Sequential instruments fitted with a polychromator can provide flexibility in line selection but at the cost of speed. Instruments of this type cost more than \$200 000. Of the typical suite of elements for plant analysis, K and Na pose some problems due to their relative ease of ionisation. Some laboratories analyse these elements by flame emission AAS or using a flame photometer rather than by ICP; however, the accuracy of ICP is generally considered adequate for measuring the concentrations of Na and K found in plants.

The combination of ICP and the quadrupole mass spectrometer (MS) has resulted in an instrument (ICPMS) with extremely high powers of detection for most elements. Typical detection limits of \leq ng/mL, good precision, multi-element capability, and the ability to perform isotope abundance and isotope dilution analysis make ICPMS a powerful analytical tool. Choice of isotope is important to minimise undesirable molecular interference, and although this can be corrected for, it may be at a cost to detection limits. With a detection limit of <0.01 Mo μ g/g, ICPMS is one of the most reliable techniques for analysing Mo in plants, as well as Co, lead (Pb), and cadmium (Cd). Typical throughput is about 20 solutions/h. Capital cost is about \$400 000, which, in conjunction with higher operating costs than comparable techniques, makes ICPMS expensive for multi-element runs for the major plant elements.

The advantages of ICP–AES discussed above make the technique very attractive for plant analysis, and this is reflected in the number of laboratories using the technique. Twelve of the surveyed laboratories (57%) were using ICP–AES and 1 of these was also using an ICPMS instrument. Eight of the 12 ICPs were simultaneous instruments. Of the 9 laboratories without ICP, 5 were planning to purchase an ICP this year or ICP would be their first choice if funds were available. In addition, 2 laboratories with older ICPs were planning to update these instruments in the near future.

Atomic absorption spectroscopy

Little has changed in flame AAS over the past decade other than to microprocessors and their associated software. There is a trend to placing all of the instrument control and data collection and manipulation into the software of the personal computer. This has made the use of standard methods much simpler and ensures that all of the set-up parameters are at the values determined as optimum for that analysis.

Future developments in AAS will be mainly in the operating software, particularly in quality control and data manipulation packages. Software available for modern ICPs is much more advanced in these areas, and the future should see manufacturers of AAS instruments incorporating similar software into their instruments.

While AAS is still the most commonly used method for cation analysis in plants (76% of the surveyed laboratories use AAS), the technique is restricted in throughput because of the '1 element at a time' limitation, with each element requiring separate analytical conditions, calibrations, etc. Consequently, AAS is being replaced by the newer, simultaneous techniques. It should, however, be remembered that despite these limitations AAS still provides excellent sample throughput rates because the instrumentation tends to be less complicated and easier to use, with less downtime, than XRF or ICP instruments. For example, 1 laboratory without ICP or XRF analyses 15 000 samples a year (Ca, Mg, Na, K, Mn, Fe, Cu, Zn) with a turnaround time of 4–5 days.

A comparison of AAS and ICP was carried out in an American interlaboratory study in 1987, using 6 reference samples analysed by 22 laboratories (Sterrett *et al.* 1987). The results indicated that AAS and ICP produced effectively the same results for Ca, Mg, Mn, and Zn, but that results for Cu and Fe were significantly higher when analysed by ICP. Potassium determinations by ICP and flame photometers showed good agreement, whereas AAS produced significantly lower values. This effect was attributed to the recognised problem of high ionisation of K under absorption analysis.

Graphite furnace (GFAAS) has been used for the determination of Co and Mo in plant tissue with varying degrees of success. Apart from the question of accuracy and precision for Mo analysis, GFAAS has many disadvantages for routine work, such as slow throughput (5–10 samples/h), labour-intensive sample pretreatment (traditionally acid digest followed by organic extraction), and high operating cost, principally for frequent replacement of furnace tubes (i.e. Mo). As laboratory microwave digestion systems are developed, the 'front end' chemistry will be simplified by making possible rapid digestions that can be analysed directly by GFAAS without extractions. To this end, the Zeeman correction system for GFAAS with its greater ability to account for background interference would be advantageous over deuterium correction, requiring less sample clean-up before instrumental analysis. Recent advances in the design of the Zeeman furnace may also improve the reliability and tube life during analysis. Despite these developments it is probable that from a cost perspective, most laboratories will still prefer a colorimetric method for Mo analysis.

Cadmium and Pb in plants are readily determined by

most GFAAS systems but can also be carried out by flame by dry ashing large sample masses to attain detection limits commensurate with specified maximum permitted concentrations in fresh fruit and vegetables.

The survey indicated that GFAAS is not widely used in plant analysis, with only 7 laboratories using the technique. Elements determined by the technique, and the number of laboratories analysing for that element, were Mo (2), Al (2), Co (2), Pb (2), and Cd (4).

Kjeldahl digestion

The Kjeldahl method has probably been used in more laboratories around the world and has probably had more modifications than any other. Today, laboratories have digestion block heaters and automatic units that can distil and titrate a sample within 3 min, while others prefer automated colorimetric methods using continuous flow analysers.

Results from an American interlaboratory study (Sterrett *et al.* 1987) indicated that the greatest agreement between 26 laboratories analysing 6 reference samples was obtained for Kjeldahl N determinations, with 80% of values within 5% of the mean. In comparison, only 70, 46, and 30% of results were within 5% of the mean for the determination of P, K, and S, respectively. These results indicate the high precision obtained in Kjeldahl analysis compared with techniques used for the other major nutrients.

Despite widespread use, there are still misconceptions about what the method measures, and it is a good example of the need to evaluate analytical quality assurance (AQA) procedures carefully. Factors such as digestion time, rate of heating, maximum temperature, ratio of acids present, and catalysts can produce variable results from either incomplete reduction of N to ammonium or N losses.

Some laboratories have abandoned the Kjeldahl method in favour of combustion analyses using a modified Dumas method. Reasons for this include concerns about disposal of sulfuric acid wastes, toxic catalysts, and, of course, safety and liability for technicians working with these chemicals. A limitation of combustion methods is the restriction of sample weights, about 100 mg, and the associated problems of homogeneity. However, N analysers that accept 500–1000 mg samples are overcoming this limitation.

Nitrogen analysers are easily automated with autosamplers and computer control to allow unattended operation after working hours. Such instruments have a total determination time of about 3 min and therefore meet the demands of rapid sample throughput and quick turnaround time.

The Kjeldahl and Dumas methods do not measure the same pool of N: Kjeldahl does not necessarily measure all the N bound to oxygen atoms, whereas the Dumas method measures the total N pool. As a consequence,

N analyser results are often slightly higher than Kjeldahl results, particularly for samples high in nitrate, such as petioles.

None of the surveyed laboratories used a Kjeldahl digestion procedure modified to include nitrate. Consequently, results for some samples from the laboratories using the Kjeldahl procedure will be slightly lower than results from laboratories using the combustion method. In order to include nitrate in a Kjeldahl determination, some sample pretreatment is necessary. One method using pretreatment with sodium thiosulphate solution (Dalal *et al.* 1984) is claimed to be faster than other methods and to produce >97% recovery of added nitrate.

The difference between Kjeldahl and combustion procedures is frequently not understood by clients, and laboratories should ensure that they explain which method was used and what it measures, and that the 2 methods are not interchanged when analysing a set of samples for the same client.

Another method gaining popularity for estimating N in certain samples is near infra-red reflectance analysis (NIR). This technique is discussed in detail later in this paper.

The questionnaire responses indicated a range of procedures for N analysis in the surveyed laboratories. Five laboratories used the Leco combustion system and report very satisfactory results with this instrument. The remaining 16 laboratories used variations of the Kjeldahl method. At least 3 different digestion procedures were employed, 5 laboratories used sulfuric acid–hydrogen peroxide, 3 used sulfuric acid with selenium (Se) as catalyst, and 1 used a sulfuric acid–hydrogen peroxide–Se–lithium sulfate mixture. Three different procedures were employed to measure the ammonium concentration. Fourteen laboratories used continuous flow analysers or FIA with colorimetric detection, 1 measured the colour in a manual spectrophotometer, and 3 used automatic titration systems.

Colorimetric methods

Colorimetric methods are still widely used for plant analysis, with 70% of surveyed laboratories using at least 1 colorimetric procedure. A dramatic reduction in the cost of microprocessors and electronic components used in today's instruments has produced great improvements in spectrophotometers, for little cost increase. Instruments now have various options for curve fitting calibrations, storing programmed methods and calibrations, storing and manipulating results, and transferring data directly to LIMS computers. The survey indicated that the most frequent uses of colorimetric methods in plant analysis were measuring N and P in Kjeldahl digests, Mo and B after ashing or acid digestion, and nitrate in aqueous extracts.

Electrochemistry

Apart from electrochemical titrations for Cl, electrochemical methods are not widely used in plant analysis. Results of the survey indicated that 40% of the laboratories that determined Cl used an electrochemical titration while only 1 laboratory used an ion selective electrode (ISE) for the analysis. No laboratory reported using ISEs for nitrate or any other nutrient. One laboratory reported using anodic stripping voltametry to determine Pd, Cd, mercury (Hg), nickel (Ni), and arsenic (As).

One reason for the lack of use of electrochemical methods may be the problems that were experienced with earlier equipment, particularly early model ISEs. Electrode technology has improved dramatically in recent times and the availability of low cost, stable, pocket-sized ISE meters for K, Cl⁻, and NO₃⁻, manufactured by Horiba, has seen a resurgence in interest in using ISEs in both the field and laboratories. Several laboratories, particularly those that analyse plant sap or fresh tissue are successfully using these electrodes, and 1 researcher is adapting the meters to provide simple, low cost, in-line detectors for monitoring hydroponic nutrient solutions.

Continuous flow analysers

The first automated continuous flow analyser was the segmented flow type introduced by Technicon in 1957. Most plant and soil chemists would have used these analysers at some time, with 67% of survey respondents currently using this technology. Perhaps the most common uses in plant analysis are the determination of PO₄³⁻, NH₄⁺, and NO₃⁻ concentrations. These instruments have been coupled to flame photometers to determine Na, K, and Ca. Newer, faster systems using narrow bore tubing and better pumps have been introduced in recent years; these provide sample rates up to 120 samples/h.

Ion chromatography

Ion chromatography (IC) measures plant anions and is most effective where several anions must be determined in each sample. It provides an accurate and simple method for nitrate but is not cost-effective when only a single anion is being measured. Problems with IC include the long elution times required to remove all organic anions from the column, base-line drift, difficulties in determining very low concentrations of an anion in the presence of higher concentrations of others, and poor peak shape. In addition, ion exchange columns are very expensive and, even if guard columns are used, can deteriorate quickly.

Although IC methods have been developed for multi-anion analysis of plant samples (Kalbasi and Tabatabai 1985), the technique has found little acceptance for routine anion analysis in plants, and not one of the surveyed laboratories routinely uses IC, although at least 2 had IC instruments.

A technique (capillary electrophoresis, CE) is now available that may address the major limitations of IC. This simple technique is matrix-independent and provides much higher separation efficiencies and selectivities than IC. Separation is achieved by applying a high voltage along a fused Si capillary containing an electrolyte. The sample is drawn into the capillary and the anions migrate from the negative polarity (injection side) to the positive polarity (detector side). The anions are quantified using a multi-wavelength UV detector. It is claimed that up to 36 anions can be separated in <5 min in real samples, that there are no base-line stability problems, and that low levels of 1 anion can be measured in the presence of much higher concentrations of other anions. Instrument costs are about \$60 000–80 000.

This technique may offer the first real solution to multi-anion analysis in plants. CE has been used successfully to measure sulfate in soil extracts (Jackson and Haddad 1993; P. E. Jackson pers. comm.). The technique could prove useful to any laboratory planning to offer a rapid diagnostic service based on sap or juice extracts from fresh plants. CE can also measure organic anions (i.e. organic acids); it therefore has the potential to allow commercial or research laboratories to offer a new service to meet changing client needs.

Near infrared reflectance spectroscopy

This technique has been used for some time for quantitative analysis in agriculture; for example, the use of NIR by the Wheat and Barley Boards to monitor grain protein levels, or the determination of oil, protein, and moisture in oilseeds. Not one of the laboratories surveyed used NIR for plant analysis.

In the past 2 years there has been a large increase in the number of sample presentation accessories, along with the development of a new generation of instruments and powerful computer software packages. These developments have largely removed the traditional limitations of the technique, and analytical systems of the future will rely heavily on NIR (Barton 1992).

NIR has been successfully applied to the quantitative analysis of major plant nutrients in a range of plant types. At a recent conference in Melbourne (Royal Australian Chemical Institute 1992), several papers covered applications such as total N in sugar cane, wheat, and rice, and the determination of a range of macronutrients including P, K, S, Ca, Mg, Cl⁻, and B in cereals and tree lucerne. These results highlight the potential of NIR for plant analysis. Instrument costs vary from \$50 000–110 000.

Because NIR offers ease of use, fast sample throughput, low cost per analysis, and minimum sample preparation, it is well suited to diagnostic analysis. The main disadvantages of NIR are the need to prepare calibrations for each sample matrix, including different

growth stages of the same plant, and the fact that the results are only as good as the calibration method and quality control procedures.

Robotics

In 1989, Robert Munter stated, 'robotics is the laboratory word for the 1990s, with increasing competition forcing lower costs and expanded capability we should see a rise in the use of robotics. There is no reason why robotics cannot be applied to plant and soil testing to perform tasks from grinding, weighing, digesting, extracting and filtering all the way to the actual analysis of the sample solution'.

To date there is little evidence of robotics being used in plant-testing laboratories in Australia. Not one of the surveyed laboratories used robotics, although 2 respondents indicated they would consider investing in this technology if funds were available.

Data management

With automation and multi-element instruments, the modern laboratory can quickly be swamped with data. Personal computers are already widely used to collect, control, and process data from analysers. But there is a need to bring all this data together in a manageable form, and that is where a laboratory information management system (LIMS) is needed. Some laboratories have designed their own LIMS; others have purchased 'off the shelf' systems. LIMS should have flexibility to meet changing needs of a laboratory, should meet client needs, and should not control the laboratory. How the system handles the AQA needs of the laboratory is another important consideration.

Problem analytes

In a recent survey of analytical laboratories, 40% of respondents (and 82% of those doing Mo analyses) indicated that analysis of Mo presented the greatest difficulty. Boron was rated second (i.e. 20% of respondents), followed by N, Cu, Se, Co, S, and NO_3^- , which were mentioned by only 1 or 2 respondents. About 24% of respondents did not identify any analytes as presenting undue difficulties.

Molybdenum

The problems of Mo analysis are reflected in the range of analytical methods used for this analyte. Methods used by the 11 laboratories that offer Mo tests included dry ashing and colorimetric analysis (3), dry ashing and ICP-AES (2), acid digestion (3 different digest mixtures) followed by colorimetric analysis (5) or GFAAS (1), and XRF (1). Problems cited with Mo included interfering spectral lines, time-consuming method, limit of detection, and complex sample preparation. Most problems with Mo analysis centre on poor detection limits obtained with current technology. With the possible exception of the very expensive solution offered

by ICPMS, there is little likelihood of solving this problem with our current inorganic analytical technology. One solution may be the example set by biochemists of looking at the potential of enzyme assays to detect Mo. Such a test would need to differentiate reliably between N-deficient and Mo-deficient plants at a lower cost and in a shorter time than conventional inorganic methods.

Boron

The second most difficult element, B, was determined in 15 of the 21 surveyed laboratories. In general, laboratories using an ICP method saw B as less of a problem than laboratories without ICP. The main problems cited were sample preparation and analysis times. ICP methods for B included digestion in nitric acid, nitric-perchloric acids, nitric-perchloric-sulfuric acids, or nitric-perchloric-hydrogen peroxide mixtures. Most laboratories without ICP relied on dry ashing followed by colorimetric measurement, often with FIA or a continuous flow analyser.

Calibration, detection limits and background correction

An emerging problem with the increasing sophistication of modern high technology instrumentation is the 'black box' mentality. The increasing use of computers and automation, while seemingly making instruments easier to operate, actually require a greater degree of skill and understanding of the instrument. Many instruments have routines built into the software packages that will determine detection limits (DL) and background equivalent concentrations (BEC), correct for drift, and do blank subtractions.

The real concern is how much we understand about the way the software calculates these and the implications for the data. It is easy to accept the instrument's output as correct, whereas, we must always question what the software has done and decide if we, as responsible analysts, are satisfied with both the rationale and the algorithms behind the decisions made by the program. For example, the detection limit in ICP has traditionally been calculated as 2 or 3 s.d. of the blank signal over 10 successive blank readings. Consequently, the detection limit is an instrument detection limit and does not take into account the variance due to front-end chemistry, instrument sensitivity differences on a day-to-day or batch-to-batch basis, or even the size of the background on which the noise is measured (DL v. BEC). Further, the size of the background and the associated noise may be different for samples and the standard blanks on which the DL and BEC were calculated.

The American Chemical Society (ACS) Committee on Environmental Improvement has attempted to reduce the confusion and inconsistencies in the use of the term 'limit of detection' and to provide a uniform approach

throughout the analytical field. Limit of detection (or DL) is defined as the lowest concentration of an analyte that the analytical process can reliably detect. This is arbitrarily based on 3 s.d. giving a relative uncertainty of $\pm 100\%$ at the 95% confidence level. This is defined as the limit for qualitative detection. Therefore, using 2 s.d. or a higher confidence level will increase the relative uncertainty of the measurements.

The limit of quantitation (LOQ), sometimes referred to as the reporting limit (LOR), has a relative uncertainty of $\pm 30\%$. It is defined as the lowest concentration of an analyte at which the precision of measurement will be satisfactory for quantitative determination. It is arbitrarily defined as 10 s.d. (Taylor 1987).

There is a wide range of opinions regarding calibration systems. In ICP software packages the calibration of DL and BEC is not directly related to the calibration, which raises the questions: should calibrations be forced through zero, what forms of algorithm are acceptable, should the line of best fit be used, should relative errors be the guide. Line fit programs will give greatest weight to the largest standard and least weight to the lowest, thus introducing systematic errors. However, unless the analyst wishes to recalculate results manually, the decision has, in most cases, already been made according to prevailing philosophy of the instrument manufacturer. Unfortunately, data-handling philosophy varies among manufacturers, leading to potential differences in results.

Analytical quality assurance

'A laboratory is expected to be able to specify the quality of its data in quantitative terms. This requires the existence of some degree of quality assurance (QA)' (Taylor 1987). The objectives of a QA system include assessment of errors in measurements, reduction of analytical errors to acceptable levels, reduction of the amount of work needed to obtain reliable data, and provision of a basis for the comparison of data. The extent of the QA procedure required to meet these objectives will be determined by the desired level of precision and accuracy and the degree to which the analytical process has been quantified.

Validation of methods is most commonly done via Certified Reference Standards. About 62% of the laboratories surveyed indicated the use of such material for quality assurance. These laboratories all included at least 1 secondary (control) standard prepared either in-house or by another laboratory, within each batch of samples. A further 24% run control standards in duplicate, thus enabling a range or precision estimate to be made independent of sample homogeneity. Forty-eight per cent of respondents plot mean and range (differences between control replicates) on control charts. Control charts are basic tools for quality assurance, as they provide a graphical means to

demonstrate statistical control, monitor the analytical process, diagnose problems, and document uncertainty of the analytical process.

The apparent anomaly between the percentage using duplicate controls and those using range charts may be due to confusion over the definition of the term 'range'. The survey did not canvas the acceptance criteria used by laboratories. The frequency of recalculation of control ranges varied from never to after each sequence run. A stable measurement process should not require frequent changes to control limits. A less stable process will result in wider control limits that, ideally, would narrow over time as the measurement process is optimised within the context of the desired accuracy and precision for that measurement.

In response to whether QA data should routinely be included on reports, 20% of respondents said yes, 14% said yes but only if requested, and 66% felt that QA data should not be included. Among those in favour of reporting QA data, opinions on what should be reported ranged from providing duplicates to providing control result and acceptable range and precision and accuracy data for the sample range.

All respondents felt commercially available control material was too expensive. Some indicated that the control range for some standards was too great. Availability of standards was considered reasonably good, the exception being specific areas such as tropical crops.

Fifty per cent of respondents reported no difficulty in obtaining suitable standards; of the remaining 50%, obtaining standards for heavy metals and nitrate was identified as a problem area.

'Plant-sap quick tests' for nutrient analyses

Traditionally, plant testing has relied on total leaf analysis. However, the time from sampling to recommendation can be excessive for many situations involving deficiencies. Also, the cost of routine sampling and analysis of several samples is often perceived by farmers as too high. Many growers who would use plant testing as a tool to improve productivity and fertiliser management often quote these problems as hindrances to the routine use of tissue testing.

An alternative to traditional laboratory analysis is a nutrient determination made on plant sap. The concept of sap analysis has existed for some 70 years, but the authors note that recently there has been a resurgence in interest with the release of more reliable tests and availability of reliable calibration data for NO_3^- , PO_4^{3-} , K^+ , and SO_4^{2-} .

Sap testing works best for short duration crops that are intensively managed with respect to irrigation and fertiliser needs. A grower of cereals, oilseeds, beans, peas, and vegetables and fruit that grow quickly must be

sure nutrition is adequate during flushes of growth. Even a short period of deficiency in a major nutrient will lead to yield and quality loss.

Plant sap analysis kits are available in a range of sophistication from simple test strips for single nutrients to sophisticated portable laboratory units that can test for several nutrients. Growers interested in plant sap testing should evaluate their goals and purchase the equipment needed to meet the needs; often a \$50 kit will suffice, but some growers who have the personnel could benefit from larger, more diverse testing kits.

Sap test kits appear to be most suited for mobile nutrients such as N, P, S, and K. These elements make up the bulk of nutrients applied as fertilisers to crops and also include the nutrients most often managed during the growing season. This makes sap testing for these elements particularly attractive. A good example is N management through the season with fertigation. The routine use of a calibrated plant sap test could assist an irrigation manager in making decisions regarding N scheduling for the crop.

The conducting tissue is sampled for sap testing via petioles of the youngest mature leaves for broadleaf crops and the first 5 cm of stem base for cereals. The main limitation of sap testing is that sap concentrations depend on the moisture status of the plant and, hence, the soil. Sampling should only be undertaken when the leaf is turgid and not wilting. Sap testing is best used as a monitoring tool, with interpretations based on trends rather than a 1-off sampling; trends should be compared with optimum levels at defined growth stages that have been determined from research during good seasons.

Researchers at the State Chemistry Laboratory (Victoria) have been involved with sap testing for several years. Excellent results were obtained for nitrate, initially using test strips, and, more recently, using CARDY ISE nitrate meters. These small meters are stable and robust and overcome the variability encountered with test strips. The results obtained with the meters are reliable and accurate over a wide concentration range, and this technology could well replace existing laboratory tests for nitrate in plant material.

Recently, quick tests for other nutrients have been evaluated, with promising results (State Chemistry Laboratory unpublished data). Tests evaluated were K using a Cardy ISE meter, PO_4^{3-} as the molybdovanadate complex using a hand-held comparator, and SO_4^{2-} using a simple turbidometric method. Results from these tests correlate well with total K, P, and S concentrations measured in the sap using ICP and on dried material using XRF. The tests were used on oilseed and vegetable crops from nutrient trials in 1991 and 1992. All 3 tests measured increases in sap nutrient concentrations as P, S, and K fertiliser rates increased. In some potato and canola trials, excellent yield responses

to applied P were obtained and good correlations existed between sap PO_4^{3-} levels and fertiliser response indicating the potential to use this test to identify deficient crops. There was no yield response to S and K in the trials; consequently, no correlations were obtained for these nutrients.

Sap testing is used extensively overseas, with a number of commercial services and test kits being available in America and Europe; several papers describing sap test technology have been published (Prasad and Spiers 1984; Schaefer 1986; Lyons and Barnes 1987; Handson and Sheridan 1992). In Australia, at least 3 laboratories (not surveyed) are offering 48-h service based on sap testing. Samples are collected by growers and mailed in post packs to the laboratory. Sap is extracted using a juice extractor or garlic press and analysed for NO_3^- , Cl^- , and K using ISE; for PO_4^{3-} colorimetrically; for SO_4^{2-} turbidometrically; and for Ca, Mg, and Na by AAS. Results are sent by fax or telephone to growers within 48 h. There are several commercial 'on-site' sap test services for nitrate. These services, calibrated for a range of crops, are both diagnostic and predictive and have been well accepted by growers over the last 2 seasons. A number of test kits are also available for purchase and many broadacre and intensive cropping farmers are using these tests to fine-tune fertiliser programs.

At the Goolwa workshop in 1981, the issue of quick tests was discussed (Longeragan 1981). Participants felt that these tests had a role to play in diagnostic testing and assessing fertiliser requirements and that laboratories should investigate their potential. Despite the fact that the technology has improved significantly since 1981 and that commercial tests have emerged, few laboratories have adopted sap testing as a routine analysis technique. Only 2 of the surveyed laboratories reported using sap tests for routine analysis, 1 of these infrequently. There is potential for all plant and soil testing laboratories to use these tests to provide a rapid, low cost, and sufficiently accurate diagnostic service for growers.

If laboratories do not accept this challenge, more growers will resort to purchasing test kits and doing their own tests. This may work for some growers, but lack of understanding of the chemistry involved and the limitations of this type of test may lead to increased costs and lost yield potential for some. Plant-testing laboratories share some responsibility in avoiding such problems by ensuring that they provide a testing service that meets grower needs and, hence, is a realistic alternative to growers doing their own testing.

Conclusion

The greatest challenge facing analysts over the next decade is not the choice of new equipment but, rather,

remaining focused on the role of plant testing in agriculture. As the needs of farmers change with changing economic and environmental issues, so, too, do the requirements of a plant and soil laboratory. The need to provide a fast, low cost diagnostic service that meets grower needs is more important, although less glamorous, than obtaining the latest instrument. Our existing technology, with the addition of some sap tests and, perhaps, an enzyme test for Mo, is more than adequate to provide the required service. Laboratories will need to improve their data management systems, perhaps using LIMS designed to meet their particular needs, in order to ensure adequate AQA, good data management, and, most importantly, rapid and reliable reporting of results.

Technologies for plant testing that will increase in use in the coming decade are simultaneous ICP; CE; NIR for rapid elemental analysis; perhaps robotics; and, definitely, improved software for instrument control, data management, and routine AQA. 'Quick test' technology using either fresh material or sap will have a significant impact on plant laboratories in the 1990s and beyond. This will be in response to the need for reduced cost per test, shorter turnaround times, and 'immediate results'. The results of the laboratory survey indicate the need to reduce the range of methodologies used for the same procedure in ASPAC member laboratories. This is particularly true in the area of sample preparation. There is considerable scope to standardise procedures like sample drying, to set optimum particle size for various analyses, and to reduce the number of different digestion procedures in use for the same analysis. Laboratory AQA approaches could also be reviewed to establish some level of uniformity among plant-testing laboratories in Australia.

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