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A new tissue test for accurate diagnosis of copper deficiency in cereals

By J.W. Gartrell¹, A.D. Robson¹ and J.F. Loneragan³

Copper deficiency in cereals can now be accurately diagnosed using tissue analysis.

Western Australia has made a further significant advance in trace element research in agriculture. Using new tissue analysis procedures, plants deficient in copper can now be reliably distinguished from those with very low, but adequate copper levels. This achievement is particularly important in Western Australia where many crops are classified as "low-adequate to deficient" in copper by existing methods. Application of copper to low-adequate crop wastes about $5 per hectare; however, failure to apply copper to deficient crops in extreme situations results in loss of grain worth $250 per hectare. Diagnosis of deficiency is often difficult, as symptoms specific to copper deficiency are often absent from affected plants. Furthermore, existing soil and plant tests do not satisfactorily distinguish copper-responsive situations from those not likely to respond (see references).

The Department of Agriculture, University of Western Australia and Murdoch University jointly developed the new method. While the method itself is proven, errors can occur as a result of such things as contamination in sampling and handling, plant variation in the sample, and in the calculation and presentation of the results. Despite stringent precautions, such errors do occur so total reliance on an analytical figure can lead to wrong and sometimes costly conclusions. In assessing the need for copper in any cereal cropping situation it makes good sense to use all the available relevant information. This includes expert knowledge of copper supply of the soil, crop requirements, the history of copper fertiliser use and plant symptoms. If the assessment based on the tissue analysis conflicts with other information, both assessments should be questioned. Further sampling may resolve the difference.

Use of test
The tissue test can be used in three ways:
• To diagnose whether copper deficiency is the cause of poor growth or disorder symptoms in unhealthy crops.
• To predict the likelihood of grain yield responses to copper in apparently healthy crops.
• To monitor copper levels from year to year.

The technique can therefore resolve any doubts about the adequacy of natural soil copper or the residual effectiveness of any previous copper fertiliser, in any cereal growing situation in Western Australia. Essential for the accuracy of the method are:
• No copper contamination in sampling and handling.
• Selective sampling of young green tissue only (the youngest fully-expanded leaf).
• Examination of plants individually to allow detection of an incidence of copper deficient plants among those of higher copper status. However, because most laboratories cannot economically analyse with the required accuracy, the minute critical amounts of copper in the single leaf of a cereal plant, a bulk sample becomes the only alternative.

For the bulk sample, tissue should be taken from 10 identical plants from the smallest, most uniform patch of soil possible. This introduces a potential source of error, but there will be no actual error if all 10 plants providing the sample tissue have the same copper status.
• Analytical procedures and methods must be able to detect 200 ± 20 ng of copper in the sample.

Time of sampling
The best time to sample depends on the purpose.
• Diagnostic samples. Samples to diagnose a deficiency are taken from plants showing signs of a disorder. They are best taken as soon as the disorder is noticed, regardless of growth stage but before flowering.
• Predictive samples. To predict whether copper deficiency is likely to develop, apparently healthy plants are sampled. Predictions indicate the most likely outcome in normal situations but the actual outcome may differ.

Equipment required includes a pair of stainless steel scissors (wash in water and detergent before use), paper envelopes large enough to take a sample, a box of paper tissues and writing materials to record details.

The sample must be kept free of all soil contamination; 10 uniform plants are selected from a small, uniform patch of soil. After the beginning of tillering the youngest fully-expanded (top) leaf from the primary (oldest and usually biggest) tiller is taken from each plant by cutting it off at the base where the leaf joins the leaf sheath. The sample is then wrapped in tissue paper, placed in an envelope and sealed, and details are recorded on the envelope and in a separate note-book.

When a disorder is present, the plants may range in size and in the degree affected. In this case take separate uniform samples representing the range of symptoms. Usually four or less categories of plant adequately covers the range. A uniform sample of plants from the nearest healthy patch of the same crop should then be sampled for comparison with the unhealthy plants.

It should be remembered that copper deficient plants mixed with copper adequate plants will not be detected if bulked in the same sample. Therefore to characterise a paddock, collect separate samples from several locations within each paddock.
The authors inspecting a part of a wheat paddock likely to be copper deficient, before sampling for analysis of the youngest emerged leaf. The suspect area is a gravel ridge of 10 ha; copper-adequate loamy soils occupy the rest of the paddock on a farm developed without copper fertiliser in the Great Southern.

tract distinguishable from others by soil type, crop growth or other features.

After sampling, keep away from possible contamination. For example, do not use copper-plated staples to seal the sample packet. Before the sample is dispatched to the laboratory, it should be dried overnight in an oven at 65 to 80°C. This can be achieved in a slightly warm domestic oven. Samples should be placed in a single layer with space for air circulation between each.

Details of the sample should also be forwarded to the laboratory. Such details include variety; planting date; sampling date; growth stage; conditions before sampling; apparent health of the crop and anticipated yield if normal conditions are experienced from sampling time to maturity; soil type; fertiliser used, past and current, especially type, rate and year of any copper fertiliser; any recognisable disorders; extent and pattern of patches of affected crop; and sprays applied. The paddock and property should also be identified with the name and address of the sampler.

The authors inspecting a part of a wheat paddock likely to be copper deficient, before sampling for analysis of the youngest emerged leaf. The suspect area is a gravel ridge of 10 ha; copper-adequate loamy soils occupy the rest of the paddock on a farm developed without copper fertiliser in the Great Southern.

The closer the sampling time is to grain development, (and therefore yield loss) the more accurate is the prediction. This is because there is less opportunity for deviations from normal development, or for plant copper status to fall as vegetative growth outstrips copper uptake. Sampling to predict a deficiency is therefore best delayed as long as possible before flowering. However, to apply a corrective spray before flowering, sampling must be early enough to allow time for analysis and for the copper spray to be applied.

Sampling at "ear-pee" is usually a satisfactory compromise, but this depends on how quickly the analysis and any necessary spraying with copper can be done. The need for additional copper applications for future crops can be predicted from tissue analysis of a current crop, knowing the behaviour of plant-available copper in soils, and requirements of possible high yielding future crops. Obviously the needs of future crops cannot be predicted with as much certainty as those of the sampled crop, but any remaining uncertainty can be eliminated by monitoring future crops. This will allow revision of gauged needs well before soil copper supply becomes inadequate.

Monitoring samples. Samples may also be taken from healthy, high yielding crops to monitor copper supply over the years. The beginning of flowering is the best time to take samples for monitoring because this is an early stage in grain development, allowing little opportunity for further reductions in the plant’s copper status.

Sampling procedure

In preparation for sampling, clothing should be free of copper contamination and hands should be washed thoroughly in water with detergent and dried on paper tissues before handling sampling equipment or samples.

Interpretation of results

Results of the analysis can be interpreted from the Table (see over). It should be noted that this interpretation does not necessarily apply where copper fertiliser has been used with the current crop. Plants within a metre of drill row vary greatly in copper status if copper fertiliser has been drilled with the seed, and it is impossible to select a uniform sample. In a bulk sample copper-adequate plants may lift the copper level of the whole sample to a level typical of an adequate plant, hiding the presence of deficient plants.

References

### Interpretation of results of tissue analysis of cereals for copper status

<table>
<thead>
<tr>
<th>Apparent health and growth stage</th>
<th>Copper level in the youngest-fully-expanded-leaf (ppm)</th>
<th>Interpretation and action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIAGNOSTIC SAMPLES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unhealthy plants. From seedling stage to start of senescence</td>
<td>Less than 1.3</td>
<td>Severe copper deficiency, spray immediately and drill copper with the next crop.</td>
</tr>
<tr>
<td></td>
<td>1.3 to 2.0</td>
<td>Lack of copper is not yet affecting plant health but a deficiency is likely to develop, particularly if improvement in other conditions limiting growth allows increased growth, and under high nitrogen supply. Spray if growth conditions are improved, and drill copper with the next crop.</td>
</tr>
<tr>
<td></td>
<td>2.1 to 3.5</td>
<td>Not copper deficient. If sample is taken from the crop in an advanced growth stage (after ear peep) the likelihood of a late improvement in growth sufficient for dilution of copper to deficient levels is slight. If the sample is taken from the crop before ear peep, copper deficiency may develop if growth rate improves markedly. Sample again at ear peep to check whether copper levels have declined to warrant spraying. Sample future better-grown crop at jointing stage of development.</td>
</tr>
<tr>
<td></td>
<td>Above 3.5</td>
<td>Not copper deficient. Even with marked improvement in growth, copper is unlikely to decline to deficient levels but this can happen if severe growth limitation is removed. Check future better grown crop between jointing and ear peep.</td>
</tr>
<tr>
<td><strong>PREDICTIVE SAMPLES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy plants. From late boot stage to ear peep</td>
<td>1.3 to 1.6</td>
<td>Severe copper deficiency will develop with normal finishing conditions (a typical &quot;purple stem&quot; situation, see Farmnote) Spray immediately and drill copper with the next crop.</td>
</tr>
<tr>
<td></td>
<td>1.6 to 2.1</td>
<td>Copper deficiency may develop in favourable finishing conditions and moderate to high nitrogen supply (another typical &quot;purple stem&quot; situation). Treat as for severe deficiency to eliminate risk of severe grain loss.</td>
</tr>
<tr>
<td></td>
<td>2.2 to 2.6</td>
<td>Not copper deficient. The only possibility of developing copper deficiency and warranting a spray is where a great improvement in growth conditions occurs (such as severe drought at sampling followed by good rains and finishing conditions) with high nitrogen supply. Sample future better-grown crops at ear peep.</td>
</tr>
<tr>
<td></td>
<td>Above 2.6</td>
<td>Not copper deficient. Any slight risk of copper deficiency is confined to future crops several times better than this crop, unless soil copper availability is reduced through unusual circumstances such as erosion or dumping several tonnes per ha of straw or other organic matter.</td>
</tr>
<tr>
<td><strong>MONITORING SAMPLES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy plants. From the start of flowering</td>
<td>1.3 to 1.6</td>
<td>Severe copper deficiency is likely to develop (a &quot;purple stem&quot; situation). Spray if time permits and drill copper with the next crop.</td>
</tr>
<tr>
<td></td>
<td>1.7 to 1.9</td>
<td>Copper deficiency may develop in unusually favourable finishing conditions and high nitrogen supply and is likely in future high yielding crops. Spray, if time permits and drill copper with the next crop.</td>
</tr>
<tr>
<td></td>
<td>2.0 to 2.4</td>
<td>Not copper deficient, but better-grown future crops might develop copper deficiency. Sample future better-grown crops at ear peep or drill copper with next crop to ensure adequate supply.</td>
</tr>
<tr>
<td></td>
<td>Above 2.4</td>
<td>Not copper deficient. Any slight risk of copper deficiency is confined to future crops several times better than this crop unless soil copper availability is reduced through unusual circumstances. Continue monitoring future well grown crops particularly if this sample level is below 3 ppm copper.</td>
</tr>
</tbody>
</table>

**Notes.**

The spray mentioned in the table is a foliage spray of 1 kg/ha copper sulphate. It must be used with caution as copper sulphate is corrosive for any susceptible metallic part of spray equipment.

Details of rates and types of copper superphosphate mixes appropriate to different West Australian soils are given in a Farmnote available from the Department of Agriculture.

*CSBP advise that farmers wishing to use this method can do so through the company's field officers.*