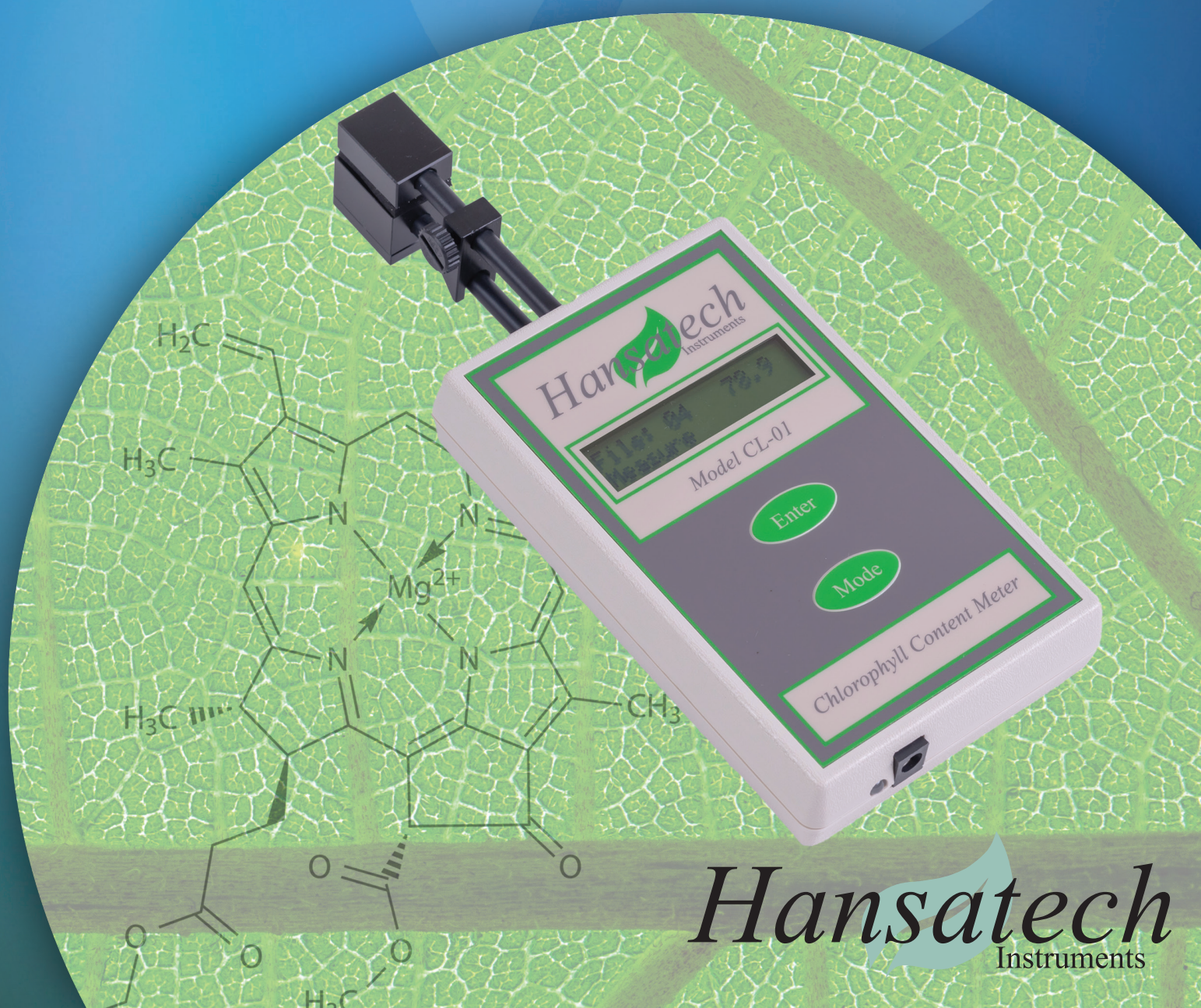


Using a chlorophyll content meter in agriculture & agronomy

A CL-01 Users Guide



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Introduction to this guide

This Hansatech Instruments application note discusses practical applications of chlorophyll content (CC) meter readings as a tool in site-specific nitrogen management.

It explains:

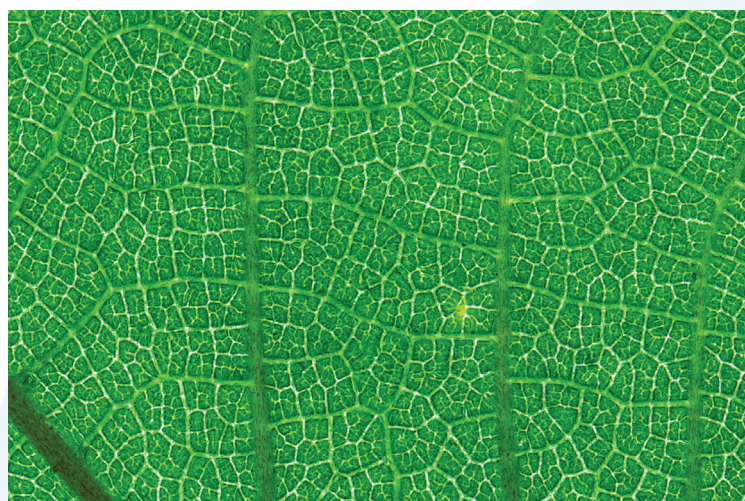
1. how chlorophyll content is related to nitrogen status
2. how a Chlorophyll Content meter works and what it measures
3. how this information can be put into practice to manage nitrogen application



The guide draws on published scientific literature and other industry-based research. It provides information relevant to using a handheld chlorophyll content meter (HCCM) in planning crop management, and in particular, nitrogen management.

Numerous studies have shown the benefit of using CC measurements in nitrogen management. For example, Singh et al (2002) noted a 12.5 - 25% reduction in N use for rice with no loss in yield, through use of HCCM measurements.

There are many variables that will affect decisions about crop management, and any such decisions can only be made in the context of local conditions and other available information about the crop in question. However, we hope the information here will offer techniques for fine-tuning site-specific nitrogen management, using rapid and non-destructive measurements from the CL-01 meter.



Introduction to chlorophyll

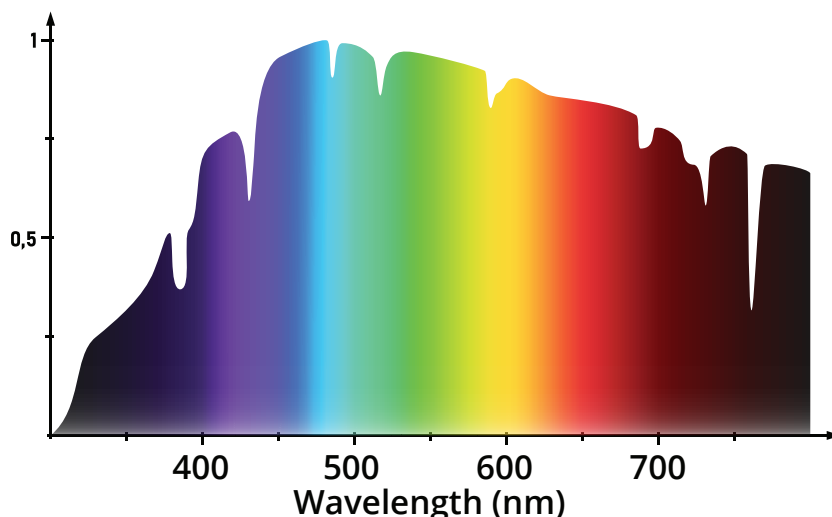
Chlorophyll is the pigment in green plants that captures light energy and enables photosynthesis to take place. Photosynthesis provides plant cells with the energy needed for growth and other metabolic activities.

Strictly speaking there are two main types of chlorophyll molecule, chlorophyll *a* and chlorophyll *b*. Chlorophyll is mostly contained specialised structures within the leaf, called chloroplasts.

Sunlight and the solar spectrum

Sunlight contains light of a wide range of wavelengths: different wavelengths correspond to different colours of light that make up the spectrum. Seen together, these appear to the human eye as white light.

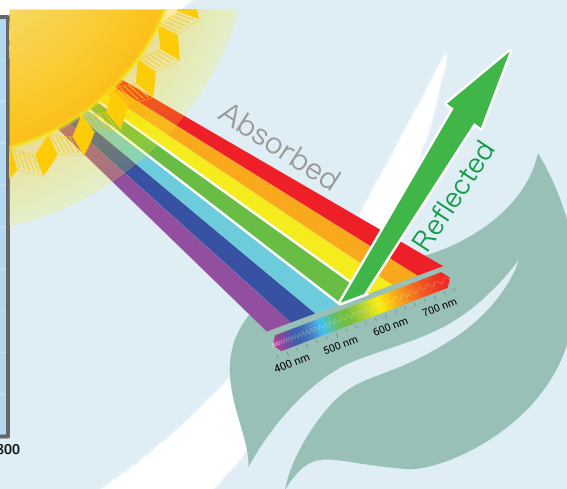
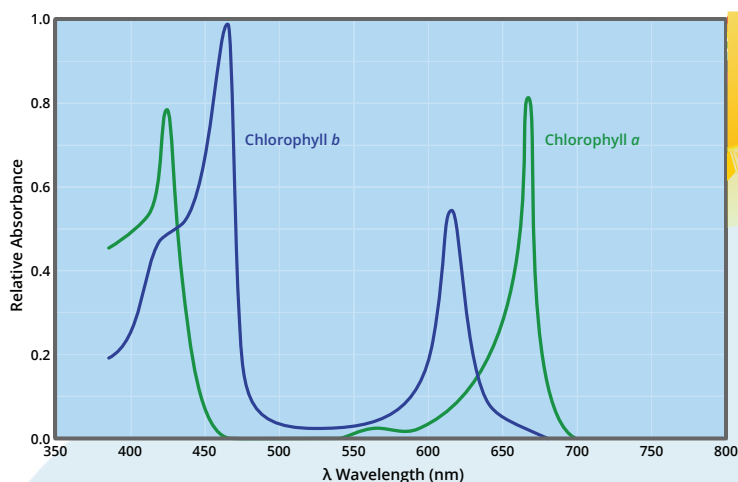
The different colours can be separated out by a prism – or by raindrops, which creates a rainbow.



NEED TO KNOW



Wavelengths of visible light are measured in nanometres (nm), where a nanometre is one billionth of a metre. Light with a shorter wavelength (ie. a lower number of nm) has more energy than light with a longer wavelength.

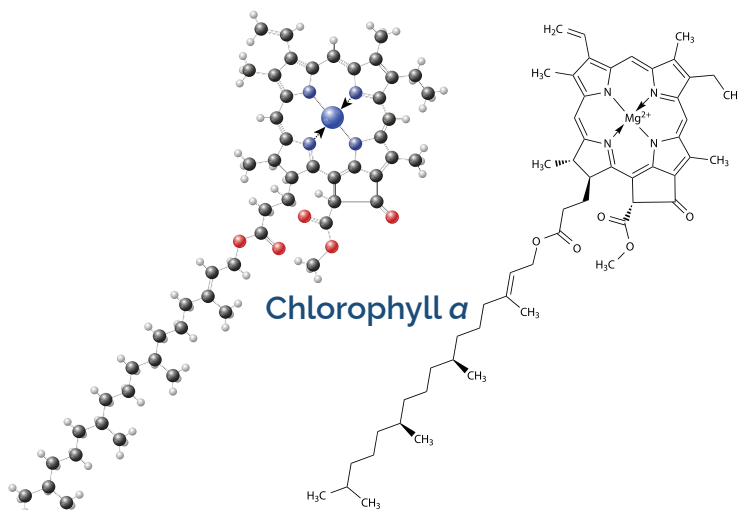


When sunlight falls onto a leaf, chlorophyll absorbs (and then uses the energy from) certain parts of the spectrum. Chlorophyll mainly absorbs blue light (peaking around 430 - 450 nm) and red light (peaking around 640 - 660nm), as shown in the absorbance diagram above. Green light (around 495 - 570nm) is reflected, making plants appear green.

Chlorophyll content and nitrogen

There is a close link between chlorophyll content of leaves and the nitrogen status of a plant as chlorophyll contains substantial amounts of nitrogen. Each chlorophyll molecule consists of a magnesium ion surrounded by a ring of 4 nitrogen atoms. Up to 75% of a plant's nitrogen can be located in its chloroplasts.

Measurement of chlorophyll content can therefore improve a grower's knowledge of crop leaf nutrition, predominantly nitrogen, and also plant health in general. It is, however, recognised that the relationship between nitrogen and chlorophyll content varies by plant species, growth stage, environmental conditions (temperature, moisture, light levels) disease, salinity and nutrient deficiency (other than N).



Measuring chlorophyll content

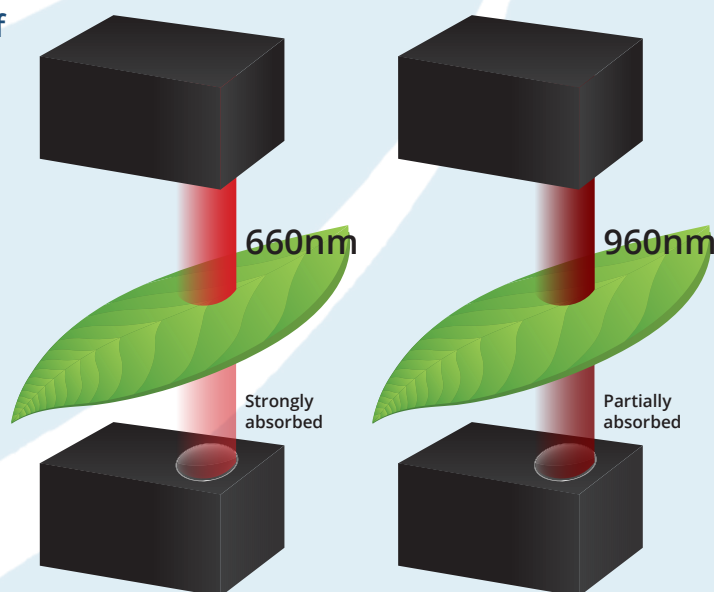
One way to measure chlorophyll content is to extract the chlorophyll from a known amount of leaf tissue in a laboratory. This can give a precise measurement of the absolute amount of chlorophyll per unit area of leaf. However, this is a destructive, time-consuming and costly process.

A handheld Chlorophyll Content Meter, such as the CL-01, can give a non-destructive, rapid and easy assessment of leaf chlorophyll content. Typically, such meters are used to monitor crop nitrogen status to help maximise crop productivity and reduce fertiliser waste. For site-specific nutrient management they are best used in conjunction with other methods, such as visual assessment; awareness of pests or disease; or measurements of soil nitrogen status.

How CL-01 measures the chlorophyll in a leaf

Handheld chlorophyll content meters such as CL-01 measure relative chlorophyll content – the Chlorophyll Content Index (CCI). This is different to measuring an absolute value, as laboratory extraction would do. The Chlorophyll Content Index expresses how much chlorophyll is present in relation to other plant tissue in the leaf cross section.

The measurement is made by passing two wavelengths of light through a leaf. One wavelength is red light (at 660 nm), which is strongly absorbed by chlorophyll (as in the absorbance diagram opposite).



The other (infra red light at 960 nm) is not absorbed by chlorophyll, and is only partially absorbed by other plant tissue. It therefore has a higher rate of transmission through the leaf than the red light.

By comparing the percentage transmission for each wavelength (that is, the amount of each type of light which gets through without being absorbed), the Chlorophyll Content Index is calculated.

$$\text{Chlorophyll Content Index (CCI)} = \frac{\% \text{ transmission at 960 nm}}{\% \text{ transmission at 660 nm}}$$

The higher the concentration of chlorophyll in the leaf, the higher the index. In the equation above, this is because more chlorophyll leads to less light at 660nm being transmitted through the leaf. This means the figure for transmission at 960nm is being divided by a smaller number.



Chlorophyll Content Index Units

The CL-01 measures in Chlorophyll Content Index (CCI) units which represents the ratio of chlorophyll to other plant tissue in the leaf. Other HHCMs calculate CCI with a similar method but may scale or transform the display of CCI in different ways.

For example, the well-known SPAD 502 meter (Minolta, Japan) transforms the CCI according to a proprietary equation, to output a "SPAD" value. As for other similar HHCMs, SPAD values are expressions of relative CCI and can be compared with results from other HHCMs, although the scale may be different.

Good practice for taking CC readings

General recommendations for measuring CCI in a crop (Shapiro et al. 2006)

1. Make multiple measurements within the crop; 30 readings are recommended to obtain an average
2. Sample in multiple locations, depending on the scale of the crop
3. Where relevant, measure equivalent leaves of the crop (for example, the most recent fully developed leaf)
4. Measure equivalent places on the leaf, avoiding the midrib or any damaged areas
5. Avoid plants that are obviously atypical of the crop in general
6. If practical, take measurements used in comparisons at the same time of day
7. Ideally avoid collecting readings when there is water on the leaves
8. Be aware that drought stress can distort readings.

Using Chlorophyll Content Index data

There are a number of approaches to using Chlorophyll Content data in crop management.

Many variables affect chlorophyll content. The ideal is perhaps use of multiple techniques to determine and calibrate interpretation of site-specific CCI readings for particular crops in particular locations and conditions.

Measurement over time

One of the simplest methods of using a HHCM is to take regular readings throughout the season and monitor. A decline in CCI may indicate approaching N deficiency. Conversely, where N is being applied, a plateau in CCI may indicate that the plant cannot use any more nitrogen. This observation may help in preventing over-application of fertiliser.

Measurement over area

Sampling chlorophyll content in different areas of a crop can help identify whether some areas are evidencing lower chlorophyll content than others, which may indicate localised nitrogen deficiency.

Comparison with reference strips using a Sufficiency Index

In extension of the approaches above is the use of reference plots or strips. These areas of a crop are provided with sufficient nitrogen, at which point the CCI would be expected to plateau. This provides a site-specific sufficiency benchmark. The chlorophyll content of a sample from the main crop can thus be compared to that of the reference strip using a Sufficiency Index, calculated as below:

$$\text{Sufficiency Index} = \frac{\text{Average reading from main crop sample}}{\text{Average reading from reference strip}} \times 100\%$$

Shapiro et al (2006) advocated an optimum N application regime being one that maintains a CCI no lower than 95% of the benchmark. Above this level, further application may result in limited benefit, while below it, there is increased risk of N deficiency (with 90% being cited as critical deficiency).

Hansatech Instruments

Hansatech Instruments is a British, scientific instrument company located in the UK. For over 45 years, our efforts have been concentrated towards the design & manufacture of high quality instrumentation for teaching & research in the fields of cellular respiration & photosynthesis. Our instruments are now in use in a wide range of programs in more than 100 countries throughout the world & have gained an enviable reputation for quality, reliability & excellent price/performance.

Products

Hansatech Instruments product range covers a wide range of applications in the fields of photosynthesis & cellular respiration.

We manufacture oxygen measurement systems based on Clark type polarographic oxygen sensors, chlorophyll fluorescence measurement systems for both continuous excitation & pulse-modulated measurement techniques & optical instrumentation for the measurement of sample chlorophyll content.

Support

Purchasers of Hansatech Instruments products can be assured of ongoing support & prompt & efficient attention to enquiries at all times. Customers are encouraged to register their instruments on our website which allows access to our Support Ticketing System in addition to instruments manuals & software upgrades



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Shapiro, C.A., Schepers, J.S., Francis, D.D. and Shanahan, J.F., 2006. Using a chlorophyll meter to improve N management. University of Nebraska-Lincoln Extension, Institute of Agriculture and Natural Resource