Chlorophyll Fluorescence OK, but what is it?

The attraction of Chlorophyll fluorescence as a new diagnostic tool in the detection of stress in trees has been increasing in the past couple of years. But what actually *is* chlorophyll fluorescence and how can it help today's consultant?

Paul Davis of Hansatech Instruments explains where this measurement technique came from and how it has evolved.

Thanks to the stimulating and informative talks given by Dr. Glynn Percival in recent years, there are few people in the Arboricultural Consultation industry that haven't heard of Chlorophyll Fluorescence and how effective it can be in detecting tree stress long before any visible signs of decline. But what actually is chlorophyll fluorescence?

Leaves can essentially be likened to photovoltaic panels in that their main purpose is to take energy from the sun and convert it to a different form of energy; in this case, complex carbohydrates that the tree may use for nutritional value. This is the main principle of photosynthesis.

No system is entirely perfect. The photosynthetic mechanisms within the leaf are unable to convert all the energy they receive from the sun into food. Consider this - on a hot sunny day, you or I would move into the shade if the sun's rays became too intense. Obviously, a tree can't do this so there has to be a method of dissipating the energy that simply can't be used for photosynthesis. A leaf has 2 outlets for the solar energy it receives:

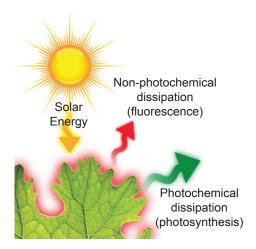
1) Photochemical processes

Photochemical processes channel the sun's energy through the necessary steps required to drive photosynthesis. A good, healthy tree is able to use about 80 - 84% of the sunlight received at the leaf surface for photosynthesis.

2) Non-photochemical processes

Non-photochemical activities dissipate energy from the leaf in a manner that is not involved with driving photosynthesis. Dissipated energy is re-emitted from the leaf in the form of infra-red radiation (heat) or far-red radiation (chlorophyll fluorescence).

Let's take an example; a period of drought causes a reduction in the rate of photosynthesis. The tree is able to convert a reduced amount of received solar energy by photosynthesis which results in a necessary increase in energy



dissipation by chlorophyll fluorescence (see diagram above).

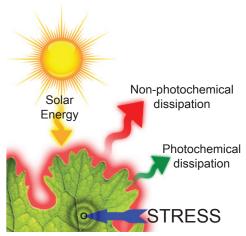
Any forms of biotic or abiotic stress which have an effect on the photosynthetic capacity of the tree will therefore alter the intensity of the chlorophyll fluorescence emission. Consequently, we can use a Chlorophyll Fluorimeter to measure changes in the extent of fluorescence emission to infer information about the efficiency of light use for photosynthesis.

In essence, the chlorophyll fluorimeter is superbly positioned to quickly and effectively screen for tree deterioration due to a stress related reduction in photosynthetic capacity. What's more, the technique is fast, non-invasive, non-destructive and can be repeated regularly.

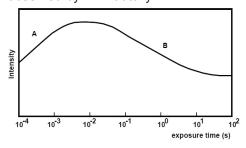
Method Behind the Madness.

The techniques and methodologies behind the measurement of chlorophyll fluorescence aren't by any means, new.

In 1931, Dr. Hans W. Kautsky (an assistant professor at the Chemisches Institut der Universität in Heidelberg, Germany), noticed that there were characteristic changes in the chlorophyll fluorescence from a leaf when it was subjected to different light intensities. These changes were termed fluorescence induction or the Kautsky Effect.



Dr. Kautsky's preliminary research on fluorescence induction was performed using the naked eye which was no easy task as chlorophyll fluorescence exists in the far-red spectral band at the upper limit of the eye's ability to see (photoptic response). The diagram below shows a graph of the intensity of chlorophyll fluorescence over time as originally observed by Dr. Kautsky.



Fortunately, from the late 70's / early 80's the electronics industry began to expand rapidly which afforded scientists the luxuries of "hi-tech" instrumentation developed by specialist companies that could measure the Kautsky Effect more efficiently and with far greater detail.



Prof. R.J. Strasser's fluorescence measurements, California - circa 1974

Birth of the PEA

Hansatech Instruments developed the Plant Efficiency Analyser (PEA) Mark 1 in 1989. The PEAMk1 was groundbreaking in that no other instrument available had ever been able to measure the Kautsky Effect with such high resolution.



PEA Mk1, 1989

Measurements from the PEA revealed detailed information about the Kautsky Effect which was previously undetected. This lead to a greater understanding of photosynthesis and how various factors could inhibit different parts of the photosynthetic mechanisms.

The PEA Mk1 and subsequent PEA Mk2 were enormously successful being employed in photosynthesis research projects the world over.



George Mackintosh of the Montserrat Impact Assessment Team studying the effects of volcanic activity on trees (circa 1996)

Further advances in technology ensued and before long, the PEA Mk2 was launched followed later by the Handy PEA (2000), Pocket PEA (2007) and more recently, the M-PEA. All the generations of PEA instrument employ the same principles of measurement.

The instruments work by illuminating a dark adapted leaf (zero photosynthetic rate) with an enormously bright red LED typically for 1 second and recording the chlorophyll fluorescence signal that is emitted during this illumination.

When the LED is switched on, the leaf instantly (time 0) begins to fluoresce as the photosynthetic apparatus of the leaf begins receiving light energy. This fluorescence intensity rises rapidly to a maximum level which, in a healthy tree, occurs at approximately 300 - 500 milliseconds. At this point, the photosynthetic apparatus in the leaf is at its maximum photochemical capacity.

Readings at the minimum and maximum chlorophyll fluorescence levels allow us to calculate the maximum photochemical efficiency of the tree according to it's current state of health. In parametrical terms, this maximum efficiency is referred to as Fv/Fm. The terms within this parameter are:

Fluorescence value at time 0 = Fo Maximum fluorescence value = Fm Difference between Fo and Fm = Fv

Fv/Fm is presented as an index value with the maximum value of 1.00 equating to 100% maximum photochemical efficiency. In reality, the maximum value of Fv/Fm we would expect to see from a tree in really good health would be between 0.80 - 0.84 which you may remember from earlier in this article, equates to a maximum photochemical efficiency of between 80 - 84%.

Fluorescence and Arboriculture

So how does all this help the Consultant Arboriculturalist in the field? Chlorophyll fluorescence has the ability to very quickly and easily, determine the photosynthetic capacity of a tree according to it's current state of health.

A reduction in photosynthetic capacity is the first stage in tree deterioration. There are many possible contributing factors to a reduced photosynthetic rate and almost all of them will have put the tree into decline long before we have visual symptoms in the form of leaf yellowing/necrosis or crown/branch dieback.

The 80 - 84% mentioned above gives us a clearly defined benchmark to which we can relate new results and, since the scientific principles behind all this are species independent, the fluorimeter is clearly a powerful diagnostic tool.

The Pocket PEA is probably the most suitable fluorimeter for Arboriculture in terms of usability and cost. With exposure at recent AA meetings and Barcham seminars, it's popularity and status continues to grow within the industry.



Pocket PEA is already used for quality control screening in nursery applications

Pocket PEA is a dedicated screening tool that bridges the gap between commercial application and scientific research.

It takes measurements quickly and effortlessly and transfers data to a PC via Bluetooth. For added flexibility, Pocket PEA can transfer data to a PDA or Smartphone running Windows Mobile 6 so that recorded data can be viewed whilst on-site for fast detection of potential issues.

The flexibility of the recorded data means that potentially, other widely used tools such as i-Tree (www.itreetools.org) and the Ashtec MobileMapper 100 (www. ashtech.com) can accept chlorophyll fluorescence data relating to individual trees to be stored alongside their own data entries.

In Summary...

Chlorophyll fluorescence is a tried, tested and accepted method of detecting reduced photosynthetic capacity due to biotic and abiotic stress factors.

Consultant Arboriculturalists do not need to be fluent in the scientific aspects of fluorescence, particularly with the wealth of talented researchers in plant physiology the UK has to offer. Already, there are large scale cooperations between Consultants and researchers such as Glynn Percival and Mark Johnson and there is no reason at all why this cannot expand to involve researchers from other universities.

So, with all this in mind, should not a chlorophyll fluorimeter be part of your toolbag?

