Answering a few practical questions about chlorophyll fluorescence

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Over the past few years the concept of measuring and/or quantifying the health of trees planted into our urban landscapes has become more common amongst professionals involved in tree management.

The reasons for this are two-fold. One, trees planted in urban or amenity landscapes (streets, town centres, car parks etc.) face a number stresses, such as drought, compaction, de-icing salt soil contamination etc., that singly or in combination can have detrimental impacts on the survival of newly planted trees and established mature trees. Two, trees under stress are more susceptible to pest and disease attack. Tree deaths equate to financial losses that are of increasing importance given the decline in resources allocated to tree management and the pressure to deliver services at lower costs. Of further concern is the fact that mature tree responses to prolonged stress include branch shedding, crown collapse and increased susceptibility to windthrow: all undesirable in densely populated areas.

For these reasons tests of tree vitality are valuable because of their ability to identify low vigour trees that will perform poorly when planted into the landscape, as well as offering the potential to quantify damage to mature established trees. Accurate measurements of tree vitality can also aid in the decision-making process to determine the economic feasibility of applying remedial measures.

Although a range of tree vitality analytical systems are commercially available to practioners involved in tree management, such as leaf chlorophyll or SPAD meters to estimate foliar chlorophyll concentrations or root/shoot electrolyte leakage to measure cellular membrane damage, one system that has attracted recent interest is chlorophyll fluorescence.

Chlorophyll fluorescence

The key principle of chlorophyll fluorescence from a health quantification point of view is that photosynthesis is one of the first plant physiological processes to be affected by stress. Indeed, photosynthesis is regarded as occupying the central position within plant biosynthesis that provides a link between the internal metabolism of a tree (tree health) and the external environment. As

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the first symptoms of environmental stress are manifest by reduced photosynthetic rates, assessing the health or integrity of the internal apparatus driving the photosynthetic process, i.e. the thylakoid membrane, provides a rapid technique for quantifying tolerance to stress. Assessing the health of the leaf photosynthetic system can be achieved by the use of CF. From a practical point of view, CF measurements are determined using a lightweight, portable piece of equipment which requires minimal training in its use and application and no detailed scientific background knowledge. This technique is non-destructive and non-invasive and only takes seconds to obtain a reading, allowing for the evaluation of many trees in a single day. All data can be downloaded or Bluetoothed to a standard PC or laptop.

Frequently asked questions

Does CF deviate during the day and does leaf colour influence this deviation?

To answer this question, four species of maple with differing leaf colour were used for experimental purposes. 45-litre containerised trees were donated by Barcham Trees, Ely:

Acer cappadocicum 'Aureum' (yellow)
Acer platanoides 'Crimson King' (red)
Acer pseudoplatanus (green)
Acer platanoides 'Drummondii' (variegated)

CF values were taken hourly from 06.00 to 22.00 from 20 leaves per tree randomly selected throughout the crown. The experiment was conducted on 25 May 2013 when the average daily temperature recorded was 16°C (an overcast day; Graph 1) and repeated on 7 July 2013 when the average daily temperature recorded was 24°C (a hot day; Graph 2).

From a practitioner's point of view, Graphs 1 and 2 show that CF values remain relatively constant throughout the day irrespective of weather conditions. Some fluctuations were recorded (ca. 5–20%) during the day, especially with *Acer platanoides* 'Drummondii', a variegated-leaf species, but nothing that cannot be accounted for by natural biological variation.

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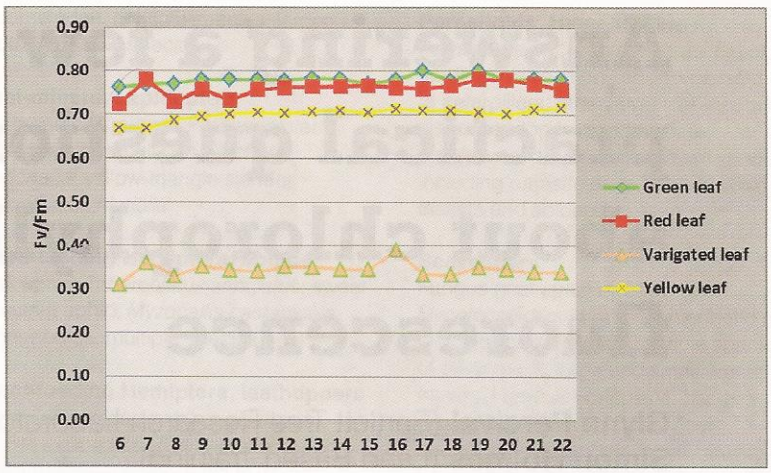
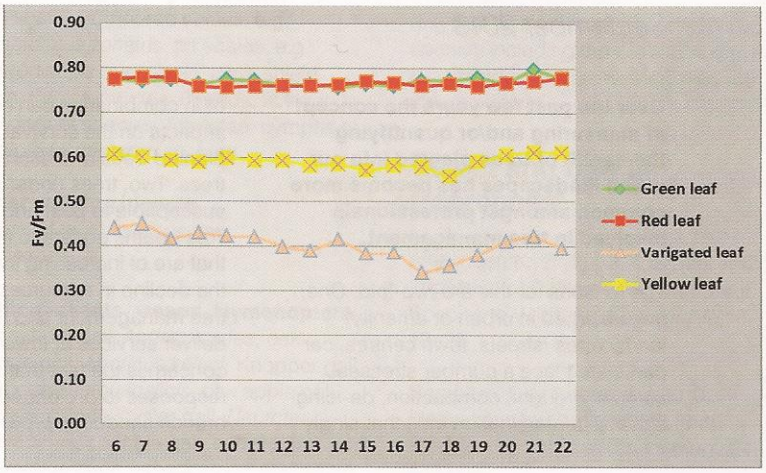


Graph 1. Chlorophyll fluorescence (Fv/Fm) over a 16h day (25 May 2013) when an average temperature of 16°C was recorded (an overcast day).



Graph 2. Chlorophyll fluorescence (Fv/Fm) over a 16h day (7 July 2013) when an average temperature of 24°C was recorded (a hot day).

With respect to red- and green-leafed maple species, chlorophyll fluorescence Fv/Fm values ranged between 0.77 and 0.81 on both an overcast and a hot day. Interestingly, in the case of a yellow-leafed maple species, chlorophyll fluorescence Fv/Fm values ranged between 0.68 and 0.72 on an overcast day but between 0.57 and 0.61 on a hot day. Such a response indicates that in this instance the maple species *Acer cappadocicum* 'Aureum' is more photosynthetically efficient on cool, overcast days compared to hot dry ones. Contrary to this, in the case of a variegated-leaf maple (*Acer platanoides* 'Drummondii') chlorophyll fluorescence Fv/Fm values ranged between 0.30 and 0.38 on an overcast day but between 0.35 and 0.45 on a hot day, indicating that this particular species is more photosynthetically efficient on hot, dry days compared with overcast ones. Such information will be valuable in improving species site selection should climatic conditions follow the consensus of models whereby the UK will become a hotter, drier place over time.

Do CF values deviate throughout the tree canopy?

It is now widely recognised that the tree canopy is a complex and dynamic, i.e. constantly changing, structure that is markedly influenced by shape, environment and pruning. Consequently the shape and physiology of leaves within the tree canopy can vary depending on their position within the crown. Leaves towards the peripheral edges of the canopy are adapted to growth in direct sunlight while those leaves that develop under the shade of other leaves, i.e. the lower canopy, are adapted to growth in diffuse light. As a result, leaves that develop in the shade (shade leaves) are anatomically and metabolically different

Figure 1. A 32m mature English oak located at Syon Park, Brentford, Middlesex, used for CF measurements.

from those that grow on the exposed canopy surfaces (sun leaves). Shade leaves typically are thinner, have more surface area and contain more chlorophyll than sun leaves and are often more efficient in harvesting sunlight at low light levels. This then begs the question, do CF values deviate throughout the tree canopy?

To answer this question several 25–35m trees of a range of species were identified. For reasons of clarity, only results for a mature 32m English oak are presented here. CF values were taken at 5m, 10m, 15m, 20m, 25m and 30m from ground level and at 1m spacings along the main limbs at each height (Figure 1). So, for example, in Table 1 a tree position identified as H5 BL1 represents readings taken at 5m above ground level and 1m along the branch length (BL). Likewise H15 BL4 represents readings taken at 15m above ground level and 4m along the branch length. At each height and branch length CF values were taken for 10 leaves.

Results in Table 1 demonstrate that, irrespective of where the leaves were measured throughout the canopy, there was little effect of canopy position. This means that readings taken from the lower canopy reflect those taken from the upper canopy peripheral edges – very useful where vitality measurements of mature trees are needed, meaning a trained climber may not necessarily be required as CF values taken from the lower canopy reflect those of the entire canopy. In addition, results demonstrated little difference in CF values between leaves measured at the canopy periphery (sun leaves) compared to leaves taken from within the canopy (shade leaves). It is important to remember that CF is a measure of photosynthetic efficiency rather than photosynthetic rates, which we know do differ between sun and shade leaves. Consequently, values show the efficiency of the chlorophyll molecules present with sun and shade leaves of English oak were comparable in this instance. Further work is ongoing to see if similar effects occur within other species.

Conclusions

Results of preliminary trials show that:

1. Chlorophyll fluorescence values of trees do not significantly vary throughout the course of the day. This indicates that measurements taken at 6.00am, for example, will reflect those taken at other times during the day.

2. Chlorophyll fluorescence values differ depending on leaf pigmentation, i.e. values for green-leaved species differ from those for yellow-leaved species etc.

3. Chlorophyll fluorescence values do not significantly differ when taken throughout the tree canopy of a mature English oak. This indicates that measurements taken from the lower canopy reflect those taken from the middle and upper canopy.

Further work is ongoing evaluating other tree species to see if such phenomena occur.

Selected literature

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Table 1. The influence of canopy position on leaf CF values of a mature 32m English oak.

Position	CF Value	Position	CF Value	Position	CF Value	Position	CF Value	Position	CF Value	Position	CF Value
H5 BL1	0.801	H10 BL1	0.800	H15 BL1	0.805	H20 BL1	0.803	H25 BL1	0.810	H30 BL1	0.813
H5 BL2	0.809	H10 BL2	0.804	H15 BL2	0.805	H20 BL2	0.814	H25 BL2	0.806	H30 BL2	0.809
H5 BL3	0.805	H10 BL3	0.799	H15 BL3	0.807	H20 BL3	0.808	H25 BL3	0.808	H30 BL3	0.804
H5 BL4	0.809	H10 BL4	0.805	H15 BL4	0.811	H20 BL4	0.809	H25 BL4	0.802	–	
H5 BL5	0.811	H10 BL5	0.803	H15 BL5	0.812	–		–		–	

All values mean of 10 leaves per position. H = height above ground level in metres. BL = branch length at each height point.
All values not significantly different from each other.