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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

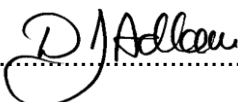
AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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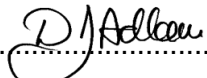
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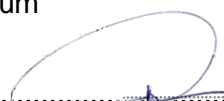
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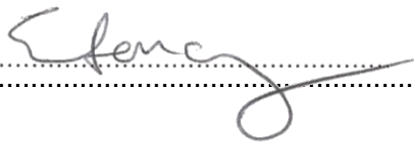
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GROWER SUMMARY

Headline

The trial aims to establish a protocol for collecting and monitoring the nutritional inputs and outputs of container hardy nursery stock growing systems. Several on-nursery hand-held pieces of equipment have been tested across a range of ornamental species and the readings compared to traditional laboratory techniques. So far the biggest problem has been obtaining on-nursery tissue or sap extracts to use with the hand-held meters.

Background

Equipment

Monitoring plant nutrient status has traditionally been done through soil nutrient and/or tissue analysis. Tissue analysis methods have been widely applied to plants due to their reliability in organic nitrogen determination, but they are time-consuming and destructive. Therefore, the focus of these studies is to test easy to use and non-destructive new tools designed for plant nitrogen (N) status estimation.

This project is initially testing five different pieces of equipment that were affordable and easy to use. The methods for sensing plant N used in this first year trial are as follows:

Nitrogen in plant sap

The extraction of plant sap is a destructive method that includes the detachment of the main petiole of the leaves, cutting it into small pieces and press it using a garlic press. As petioles in ornamental plants are mostly very small, in this trial leaf samples, including the petiole, were used and leaf sap was analysed.

Two pieces of equipment were used to measure the concentration of nitrate in leaf sap.

Merck Nitrate test strip

Nitrate test strips change colour when exposed to nitrate contained in the sample of plant sap. The colour can then be compared to a colour chart (subjective method) or be measured by a hand-held reflectometer. The Merck test strips used during this project were those for the detection of nitrate the Merckoquant NO₃. This test strip measures from 0 ppm to a maximum of 500 ppm NO₃. Merck test strips are quick, easy to use and very cheap (£30.00 per 100 pieces). Quant strip tests measure in nitrates instead of nitrate-N, therefore readings must be divided by 4.43 to find the nitrate-N value.

Horiba Laquatwin Nitrate kit

For this piece of equipment, nitrate levels in plant sap are measured using a nitrate sensitive electrode. This compact nitrate sensor has an operational range from 23 to 2,300 mg/L and only needs a few drops of plant sap to generate a reading (enough to cover both electrodes).

However, this technology does have some disadvantages:

- (1) It does not measure total N in plant tissue but only NO₃-N,
- (2) The presence of other ions such as chloride, bicarbonate or nitrite can affect measurements (b).¹
- (3) Frequent calibration is also needed to maintain the accuracy of the sensor (every 5 samples)
- (4) Readings should be made in the shade since direct sunlight can affect the meter.

Optical sensing methods

The greenness of the leaves represents the amount of chlorophyll found in the chloroplasts. Leaf chlorophyll content can be used as an N status indicator, because this is an essential element in photosynthetic protein synthesis. Leaf chlorophyll content increases with N supply and decreases when N is limiting.

atLEAF⁺

The atLEAF⁺ is a sensor that measures leaf Chlorophyll content in a similar way as a SPAD meter, but has the advantage of being cheaper. It is a non-destructive, hand-held, lightweight and easy-to-use sensor.

The device works by inserting the leaf into the aperture in the front of the sensor and clicking on the measure button. There are two LED emitters in the upper part of the aperture at two wavelengths, red at 660 nm and near infra-red (NIR) at 940 nm. Light filtered through the leaf is captured by a sensor below it which measures the absorbance of the leaf. The difference in transmission of the filtered wavelengths gives a measure of chlorophyll content in atLEAF⁺ units. The sensor continues to sample the scanned area as long as the measure button is being pressed. An average value of the measurements will appear when the measure button is released. The device can measure leaves that are up to 0.1 in (2.5 mm) in thickness. Measurements can be stored and easily uploaded to a computer.

According to literature this sensor is not very effective at collecting readings on leaves with small widths like conifers. Unlike SPAD that makes the measurements in a closed chamber which clamps over the leaf, and has a filter to clear other wavelengths in the light spectrum,

¹ This has always been a problem with ion specific electrodes

the atLEAF⁺ sensor takes measurements in an open aperture and has no filters. This is likely to affect the readings because: (1) the position of the leaf can vary (closer to the top part of the aperture or to the lower part) and (2) this aperture allows for light to reach the sensor diode and interfere with the reading.

Fieldscout Green Index App

This App was developed to capture differences in ‘greenness’ between maize leaves. The app captures images using the iPhone digital camera and determines the DGCI (Dark Green Colour Index) of plant leaves (between 0 and 1). When purchasing this App, growers should also purchase a reference board which is used as a background when taking pictures of the leaves. The green and yellow discs present on this board are known colours (standards) used by the software to calibrate differences in light conditions; the pink background increases contrast and reduces noise, and the grey colour calibrates the white balance.

Because N status is not the only factor that affects the greenness of the leaves (water stress, temperature, and cultivar also do), the DGCI readings taken in a field must be compared with readings taken in a high-N reference area. Recent studies show that DGCI is closely related to the N content in leaves as well as with SPAD readings.

Electrical conductivity (EC) in substrate

Monitoring the N present in the growing media through substrate analysis, is a method widely applied in the hardy nursery stock industry. However, sampling growing media is labour-intensive, expensive, and growers have to wait for the results in order to be able to adjust fertilisation regimes.

“Procheck” and “GS3” sensor probe

The sensor GS3 from Decagon measures soil moisture, temperature, and electrical conductivity (EC) of the substrate. The probe has three steel needles that improve sensor contact in porous substrates such as peat or perlite. By measuring EC in the substrate solution, the sensor measures the total amount of salts dissolved in pore water. It does not give information on the amount of a specific nutrient. However since the majority of salts in the substrate are macronutrients, EC can be used as an indicator of the presence of macronutrients in the growing medium.

Summary

This report covers the first 12 months of the three-year project. The aim of the project is to try to relate crop performance measured using various hand-held instruments, to traditional sampling and laboratory analysis results. Additionally, by monitoring rainfall and irrigation

inputs, we look at the nutrient balance between the inputs, uptake by plants, and outputs through leaching.

In this first year two main trial sites were established, Greenmount College in Northern Ireland and PCS Research Station at Destelbergen, Ghent, Belgium. It was anticipated that their geographic positions would give some different inputs, such as hours of sunshine, temperature and rainfall. The trials were established using the same base substrate, plant species and increasing rates of CRF fertiliser. Four commonly grown hardy nursery stock subjects were selected based on criteria such as, colour reaction to fertilisers, vigour of growth in relation to fertiliser rates, and growth habit. These subjects were *Viburnum tinus*, *Chamaecyparis pisifera*, *Skimmia japonica* and *Buddleja davidii*.

At each of the sites the rainfall, irrigation and other climate data was collected. At pre-determined growth stages, samples of plant tissue, substrate mix and runoff from specific trial plants were collected and sent for full analysis. In addition to the traditional sampling, the site operators measured parameters such as sap 'N' content, leaf chlorophyll reflectance, moisture and EC levels in the substrate using a range of hand-held instruments, and a novel mobile phone app for measuring the health of leaf tissue, all with varying levels of success.

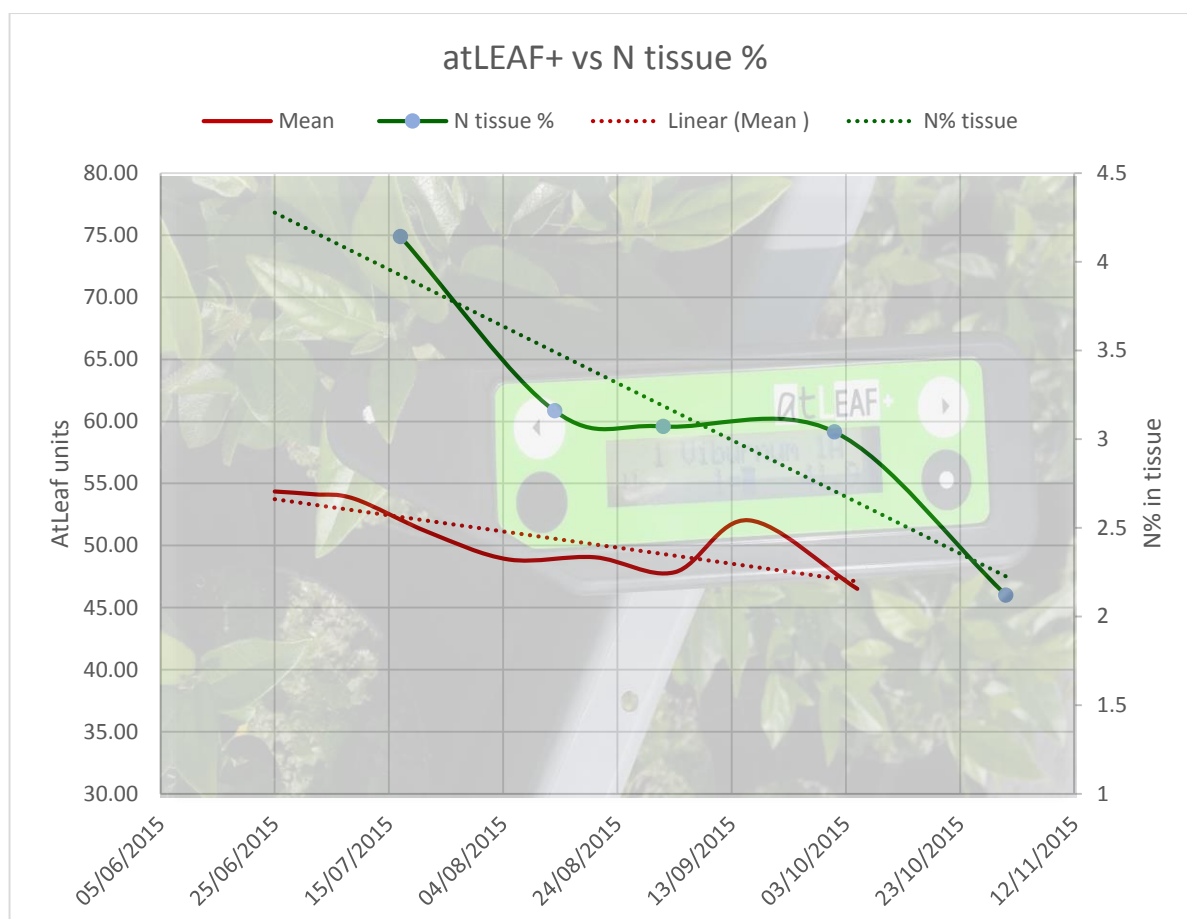


Figure 1. atLEAF+ N values compared to leaf tissue N % by analysis

A wealth of data has been collected and already shows a strong correlation between plant health and nitrogen status, using leaf light reflectance. These findings are comparable to tissue analysis (see Figure 1 and page 18 for more detail). Additionally, substrate probing techniques used on the nursery also appear to give accurate measurements of plant health and growth. One of the main problems when using hand-held instruments, is that where 'sap' is required for readings, it is very difficult to get enough sap from some of the hardy nursery stock plants tested, and the end results may be so coloured that they interfere with the reading being made. Work by the States of Guernsey advisory service indicate that this is a common problem with sap analysis, but can be overcome (Smith, 1987; Marchant-Smith, 1995). This will be looked at in more detail in the second year of the project.

Data on the leaching of nutrients from the system is closely related to the vigour of growth of the selected species, although caution is needed as some of the plants in the trial were not of good quality and hence their performance may have skewed the results.

In the second year of the project, the same two monitoring sites will be used again, and additionally J Coles and Sons Ltd. of Leicester will also host a trial. This is to hopefully give a geographical mid-site in terms of climate and growth conditions. Each of the sites will major on one plant species and take samples every two weeks, in addition to measuring the wider

range of plants. Two other growers, Osberton Grange and Frank. P. Matthews have agreed to trial specific hand-held pieces of equipment to establish whether they are easy to use.

There are also planned open days and visits to the trial sites through the autumn of 2016, and a series of hands-on workshops will be held across the winter period 2016-17.

Financial Benefits

Annual UK sales of CRF fertilisers are currently £4m (across all manufacturers) with a farm gate market value of £1b for all hardy ornamental plants. The cost of fertiliser is quite a small percentage of sales but the effect on a grower's sales when they have a nutrition problem can be a very high. We asked a group of seven nursery stock growers what the crop losses were from nutritional problems. No one was able to quantify these losses. The below is from Table 1 from the science section showing the price of the equipment assessed during the project:

Equipment	Measurement	Price
'Merck' Nitrate test strip	Nitrate – NO ₃	£30.00 per 100 tests
Horiba 'LAQUAtwin' Nitrate kit	Nitrate – NO ₃	£380.00
'Procheck' and GS3 sensor probe	volumetric water content, temperature and electrical conductivity	£730.00
atLEAF+ meter	chlorophyll concentration	£210.00
'Fieldscout' Green Index App	DGCI (Dark Green Colour Index) & SPAD (chlorophyll concentration)	£100.00 (excluding smart phone)
'Pour-thru' devices	Pour-thru water collection	£24.00 each
Davis 'Vantage PRO2'	Weather data	£980.00

Action Points

Growers must decide whether they are introducing an on-nursery monitoring system and they must then engage and train a suitable member of staff to make the measurements and develop the trends for a range of subjects.

For growers the current messages are:

- 1) Various instruments are sold for on-nursery testing but currently there is little guidance on how to achieve a data set which can be interpreted easily for hardy nursery stock,
- 2) Both substrate conductivity and leaf colour offer promising data to determine leaf N content,

- 3) The extraction of sap and leaf samples from nursery stock subjects is at best difficult and may require additional treatment before testing,
- 4) If the monitoring is to be meaningful, the time of day of sampling, the frequency of sampling and the consistency of the operator need to be taken into account.

SCIENCE SECTION

Introduction

Annual UK sales of CRF fertilisers are currently £4m for all manufacturers with a farm gate market sales value of £1b for all ornamental plants. The cost of fertiliser is quite a small percentage of sales but the effect on the sales of a grower's crop when they have a nutrition problem can be extensive. This project seeks to address and quantify nutritional deviations before they become a foliar disorder and allow the grower to rectify it in time.

In the past the nutrient status of crops has relied on 'reactive' analyses of substrate and tissue often at a point where correction is difficult. The literature review included here indicates that various hand-held pieces of equipment are now available which purport to be able to give field measures of 'N' content of growing plants. The protocols being examined in this work aim to test each hand-held instrument and the values they produce in relation to laboratory analysis, and to look for trends and similarities in results, such that these simple devices can predict trends and therefore make additional fertiliser applications 'proactive' over time.

This project aims to provide nurserymen with practical methods to monitor plant nutrients and measure plant uptake so nutrient applications can be adjusted accordingly. This is being carried out by regularly taking a series of measurements with various pieces of equipment (outlined later in this report) and comparing them with the analysis of plant tissue, growing media and pour-thru water. Weather data (air temperature, rainfall and humidity) were collected at each site.

Materials and methods

The trials were carried out in two locations with different environments in order to expand the knowledge of crop responses. The two trial sites were at PCS in Ghent, Belgium and Greenmount College, Northern Ireland. Tissue samples, substrate samples and pour-thru water samples were all analysed for major nutritional elements when a plant growth extension of 2 cm was achieved, in addition to the hand-held equipment measurements.

We asked a group of seven nursery stock growers what the crop losses were from nutritional problems and their responses have helped to clarify what areas we should target to provide the best adoption of the results found. None of the growers were able to quantify the losses due to nutritional problems, but suspected that they had high fertiliser release from CRF fertilisers when used under protection in hot summer periods. As a result of this, their CRF rates were either reduced or in one case excluded. One grower stopped growing a difficult plant. All the growers were using the plant condition as a guide to the health of the crop, but generally this was dealing with the issue after the problem had emerged. This demonstrated

to us that a method of assessing nutrient availability and uptake prior to any visual symptoms manifesting themselves would have financial benefits to the grower. Ornamental crops require blemish-free foliage and any deviation from a strong and healthy plant can render it totally unsaleable.

The same base growing medium was mixed with an 8-9 months CRF at the rates of 2, 4, 6 and 8 g/L and shipped to the two sites. In the beginning of May, 2015, young plants were potted into 1.5-litre pots and placed in groups of 20 plants in a growing bed. Six plants were placed in the pour-thru containers and the remaining plants were placed around them (see Figure 7).

'Pour-thru' devices

Low cost 'pour-thru' systems were developed using two grey and opaque plastic containers: (1) a smaller perforated container where plants were grown (60 x 40 x 21.5 cm) and (2) a bigger container underneath used to collect the drained water from the container above (60 x 40 x 32 cm). The top box had an even pattern of holes drilled in the bottom in which to allow water to pass through. Capillary matting was then added to the base of the top box and the MARA (ART NR 6015 B) plant tray of six 1.5 litre pots set into the box (see Figures 2 and 3).



Figures 2 and 3. Pour-thru units at PCS Belgium (left) and Greenmount College N Ireland (right)

Weather station

At Greenmount a wireless Davis Vantage Pro Weather station complete with solar panel attachment and wireless console was installed in the project nursery stock area. Data was downloaded using the software and USB connection suitable for Windows application. www.davisweather.co.uk



Figures 4 and 5. Wireless Davis Vantage Pro Weather station: in the field (left), and in detail (right).

Four hardy nursery stock species were used in the trial:

- *Viburnum tinus*: frequently used in HNS trials, compact and visually sensitive to nutrient levels
- *Chamaecyparis pisifera* 'Boulevard': because its foliage colour is a good indicator for its N status (important for optical sensing methods).
- *Skimmia japonica* 'Rubella': because it is an easy to assess shrub that produces plenty of succulent foliage (important for plant sap methods).
- *Buddleja davidii*: is a vigorous growing shrub and very responsive to fertilizing levels (growth and colour)

The plants in the trial were all irrigated at the same time with overhead sprinklers (Figure 6). In Belgium the irrigation was triggered by a maximum radiation sum. The radiation sum was adapted throughout the season (low value in the summer and high value in the spring and fall). The species and rates of CRF were combined in a 4 x 4 factorial design with three replications (Figure 7).



Figure 6. shows the overall view of the plant trial at PCS, Belgium

The growth of the Buddleja plants naturally became excessive and to correspond to commercial practice they were trimmed back on both sites. This is reflected in the plant height data.

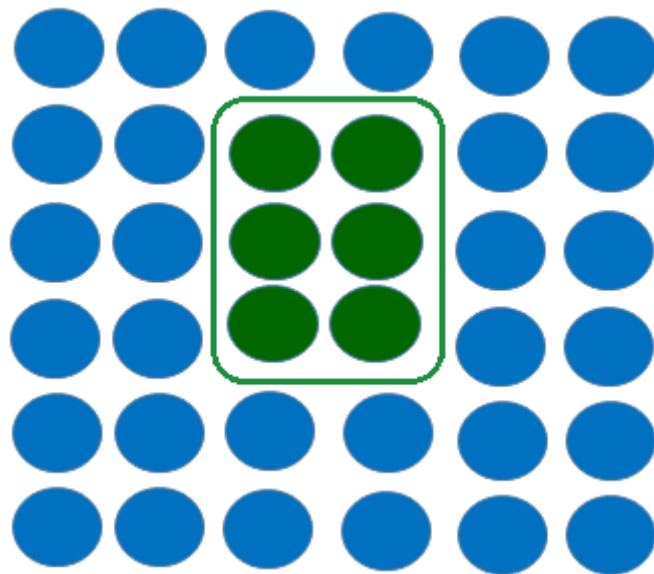


Figure 7. Schematic of the plant trial at Greenmount College, N. Ireland. The green spots represent the plants in the 'pour-thru' devices, the blue spots are the surrounding plants.

Substrate mixes used and Product Specification

Main ingredients

3 parts Dark peat 18 MM	}	80% of the mix
6 parts Light peat 18 MM		
1 part Sod peat 18 MM		20% of the mix
FOREST GOLD® -----		

Nutrients added per m³:

1 KG BASE 15-10-20+TE
0.1 KG NITROCHALK
3 KG LIME/DOLODUST
0.4 LITRE WETTING AGENT

CRF additions:

2, 4, 6 & 8 kg/m³ of 8-9 month Osmocote (standard release product)

'Pour-thru' collection and analysis:

Every four weeks, the volume of drained water in the lower container of the pour-thru was measured, sampled for water analysis and emptied. At the same time substrate samples were taken and sent to the laboratory. Samples from each site were sent to the same laboratory to avoid differences in procedures. Temperature and rainfall were measured by weather stations present at each site, and the amount of water received by the plants (rainfall plus irrigation) was measured by rainfall gauges. Run-off and nutrient leaching could therefore be monitored to give us an accurate idea of the amount of nutrients being used by the plants and lost through leaching. Plant height was measured every two weeks. Each time a plant species grew more than 2 cm, a leaf sample was taken for tissue analysis. At each site, five different hand-held pieces of equipment were used every two weeks to obtain measurements of the nutritional status of the crop. These results have then been used to compare trends of the results with the full laboratory data sets. The hand-held devices used are set out in detail below.

Nitrogen in plant sap

Leaf sampling and leaf sap extraction occurred differently at each site. In Northern Ireland leaves were sampled from mid-way on the plant and fresh sap was used, while in Belgium the first fully expanded leaves from the top were sampled and leaf material was frozen to increase the volume of extractable sap. Leaf samples in both sites were taken from the guard plants surrounding the pour-thru devices.

Statistical analysis of all data was carried out by the statistics department of CAFRE at Greenmount. There were 153 variables, of which 62 are listed of particular relevance and

detailed in Appendix 12. Each variable is tabulated to show the significance level, where “NS” is no significance, “*” shows significance where $P < 0.10$, “**” shows high significance $P < 0.05$ and “***” showing very highly significant results where $P < 0.01$. The tables are shown for each plant variety, each replicate and both sites combined. All the graphs relate to the various datasets referenced in Appendix 12 and show the degree of significance of the various data sets when plotted together. The graphs are tabulated in plant groups. (See Figures 21 – 27)

Equipment

1 Merck Nitrate test strip

Merck test strips are quick, easy to use test strips suitable for the semi quantitative detection of ions, and organic and inorganic substances. They give a quick summary of substance concentrations present in the sample.

The Merck test strips used during this project detect nitrate (the Merckoquant NO_3N). This test strip measures from 0 ppm to a maximum of 500 ppm NO_3 .

The test strips use the Azo dye method for testing nitrates. Nitrate is reduced to nitrite by a reducing agent. In the presence of an acidic buffer the nitrite is converted to nitrous acid which diazotizes an aromatic amine compound with N-(1-naphthyl)-ethylenediamine to form a red-violet azo dye (see Figure 8).



Figure 8. Merck test strips being compared to colour standards for the detection of nitrate

Greenmount

A random leaf sample (including the petiole) from mid-way on the plant, was picked early morning. The end of the petiole was then placed into a pair of wide end pliers along with one of the test strips with the test end alongside the petiole. The pliers were then squeezed releasing a small amount of sap which stained the test strip test end. After 1 minute the colour formed was then read against the colour chart supplied on the storage container. One sample was taken from each treatment and plant species, and replicated at fortnightly intervals from the six plants within the pour-thru devices.

PCS

In Belgium five to eight leaves (depending on the species) were taken from each replicate, placed in a syringe and frozen (one syringe per plant species and per treatment). Every month syringes were left two hours to thaw. After a two-way calibration, plant sap was squeezed from the syringes into the cavity of the Laquatwin NO₃ sensor and nitrate was measured. Immediately after, a nitrate test strip was dipped in the same leaf sap sample (and after one minute the colour was red as already referred above). Two-way calibration was performed after every four samples and demineralized water and absorbing paper were used to clean the sensor cavity between measurements.

2 Horiba Laquatwin Nitrate kit

Horiba scientific provides a range of bench top and portable meters suitable for use in the lab or field. The Laquatwin is a hand-held, self-contained, waterproof, compact meter available with a selection of seven parameters such as pH, conductivity and various ions (see <http://www.horiba.com/uk/>).



Figure 9. Laquatwin NO₃ sensor used for the nitrate measurement of plant sap

Greenmount & PCS

A minimum of 15 leaf samples (including the petioles) were collected in the early morning from mid-way on the plant. One collective sample was taken from each treatment and for plant species and replicated at fortnightly intervals from the six plants within the “pour-thru” devices.

All samples were stored on ice for up to eight hours before processing. Each collective sample was placed in a syringe and squeezed to form a liquid sap sample.

Following calibration of the LAQUtwin NO₃ meter the squeezed liquid sap sample was placed on the flat sensor and the reading taken. At Greenmount the sampling sheet (known as sampling sheet B part No. 3200053858) was used to counteract the influence of particles within the sample.

For the PCS the same sap sample was also used for the Merck nitrate test strip reading.

‘Procheck’ and GS3 sensor probe

Decagon Devices Inc. designs, manufactures and markets scientific instruments with a focus on measuring water, light and temperature within the soil-plant atmosphere. (see www.decagon.com).



Figure 10. (left) shows the Procheck which provides a digital reading for volumetric water content, temperature and electrical conductivity.

Figure 11. (right) shows the GS3 probe used to take the readings.

Before starting, the probe (Figure 11) needs to be given time to acclimatise to the outside temperature especially if coming from storage.

Prior to measuring the GS3 sensor has to be configured according to the type of growing media: (1) ‘mineral’ for soil and (2) ‘soilless’ for substrate. At PCS, the probe was inserted at the top of the substrate while at Greenmount it was inserted at the side after removing the plastic pot. A reading is then processed within seconds. Measurements can be saved and names can be given on site. This is better than the atLEAF⁺ device where names have to be processed by a computer program and uploaded into the sensor before taking it to the field.

All six plants were sampled within a “pour-thru” thus a total of 18 plants were sampled per plant species and treatment. Electrical conductivity, volumetric water content and temperature were also automatically registered.

Following completion of all the readings for the day the data was downloaded using the “Procheck” software and USB connection suitable for Windows application.

4. Optical sensing methods

‘atLEAF⁺’ meter

Optical sensing equipment was always used early in the morning, sampling one leaf per plant from the six plants within the “pour-thru”. It was found that depending on the species the number of leaf measurements needed to obtain a realistic and steady mean value varied. The sampled leaves were not removed from the plant during the measurements (non-destructive method).

The atLEAF+ meter is an easy to use, hand-held meter, measuring the relative chlorophyll content of green leaf plants (Figure 12). It measures the optical density difference at two wavelengths, 660 nm and 940 nm (<http://www.atLEAF+.com/SPAD.aspx>). The device works by inserting the leaf into the aperture in the front of the sensor and clicking on the measure button. Measurements were taken in the middle of the leaf ensuring always that the leaf was correctly positioned under the sensor's window. The sensor continues to sample the scanned area as long as the measure button is being pressed. An average value of the measurements will appear when the measure button is released. The device can measure leaves that are up to 0.1 in (2.5 mm) in thickness.



Figure 12. atLEAF+ meter measuring the relative chlorophyll content of green leaf plants.

Greenmount & PCS

In the early morning one leaf was selected from one plant within the “pour thru” devices and measured using the atLEAF+ meter. The leaf was not removed and any water / moisture on the leaf surface flicked off before measuring. One sample was taken from each treatment and plant species and replicated at fortnightly intervals.

By inserting the names of the different plant species, treatments and replicates, the data is saved in a clear way and the user can easily view and compare previous measurements. Following completion of all the readings for the day the data was downloaded using the software and USB connection suitable for Windows application.

5. 'Fieldscout' Green Index App

The Fieldscout Green Index App is a smart phone application designed for use on iOS devices such as an iPhone. The app uses the power of the smart phone to capture the difference in the greenness of the plant leaf. This app is only available for iPhone and captures images using the camera and determines the DGCI (Dark Green Colour Index) of plant leaves. A specific reference board has to be separately purchased and acts as a known background to the App. The green and yellow discs on the board are known colours (standards), and are used by the software to calibrate differences in light conditions; the pink background increases contrast and reduces noise (see Figure 13), and the grey colour calibrates the white balance. Before starting the measurements, the user has to calibrate the white balance over the grey area (on the reference board) and lock the camera's white balance features. The DGCI has been correlated to SPAD readings for chlorophyll concentrations. (www.specmeters.com).



Figure 13. The reference board which acts as a known background to the App used.

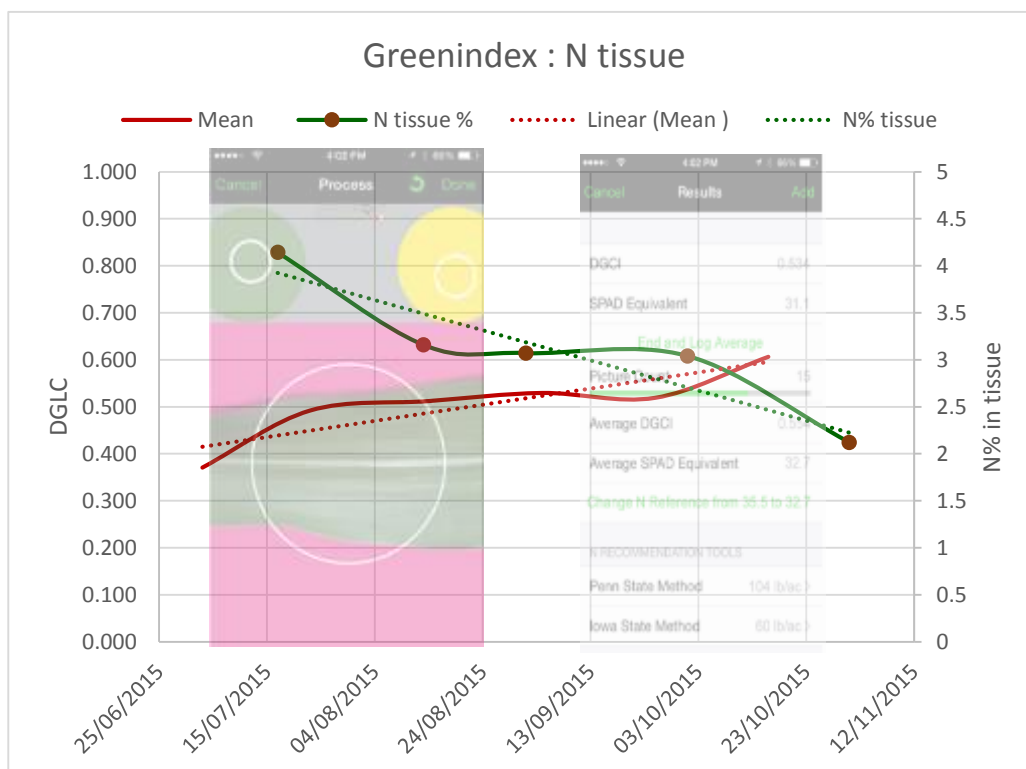


Figure 14. The comparison between the ‘Fieldscout’ measurements (the mean, shown in red) and the N tissue % from analytical data.

Greenmount & PCS

After downloading and configuring the app, one leaf was selected early morning and removed from one of the six plants within the pour-thru devices and laid on the target board. The image was captured and the readings were taken. One sample was taken from each treatment and plant species and replicated at fortnightly intervals. As each image is captured it can be shared with an email account providing logged data which can be imported into Windows Excel.

Table 1. Equipment used and assessed during the project

Equipment	Measurement	Price
‘Merck’ Nitrate test strip	Nitrate – NO ₃	£30.00 per 100 tests
Horiba ‘LAQUAtwin’ Nitrate kit	Nitrate – NO ₃	£380.00
‘Procheck’ and GS3 sensor probe	volumetric water content, temperature and electrical conductivity	£730.00
atLEAF+ meter	chlorophyll concentration	£210.00
‘Fieldscout’ Green Index App	DGCI (Dark Green Colour Index) & SPAD (chlorophyll concentration)	£100.00 (excluding smart phone)
‘Pour-thru’ devices	Pour-thru water collection	£24.00 each
Davis ‘Vantage PRO2’	Weather data	£980.00

Statistical methods applied

In 2015 the experiment (four species x four compost treatments x two sites (Greenmount & PCS)) was arranged as a block design with each block containing each of the four species and four compost treatments. Statistical analysis was carried out using statistical software by Biometrics & Information Systems, Agri-Food & Biosciences Institute (AFBI), Northern Ireland.

Data was analysed using ANOVA. A general ANOVA test between the four compost treatments with 3 degrees of freedom was carried out. Three specific tests were done: linear, quadratic and deviation with 1 degree of freedom. Any significant correlative relationships for individual residuals and between residuals for two variables are also listed. Some of the data is tabulated in Figure 21-23 for *Viburnum* and Figure 24-27 for *Buddleja*. The main effects of commercial interest are presented as line graphs within the results section.

Results

The equipment data output and data analysis clearly indicate that specific equipment showed (good) positive relationships to traditional lab analysis. However more work is needed as obtaining a sufficient sap sample from the shrub species was hard to achieve and the sample was also stained green with the plant chlorophyll making it difficult to decipher colour readings

The LAQU twin NO₃ meter was found to be simple to use, however due to the preparation required for the plant tissue sap samples, all readings were carried out under laboratory conditions, ie. away from the nursery beds. Usage of the “Procheck” and GS3 sensor probe to measure substrate properties was found to be simple and suitable for use in the nursery. The atLEAF⁺ meter was found to be easy to use and suitable for use on the nursery as long as attention to the leaf position in the ‘jaws’ of the meter were attended to. Whilst using the Fieldscout Green Index App, the methodology was very simple but required some finger agility and precision when tapping on the App screen. While less practical than the atLEAF⁺ meter, the Fieldscout Green Index App was found to be easy to use and suitable for use within the nursery, although the results do not immediately appear to correlate well with the laboratory analysis.

Equipment results

atLEAF⁺ and Green Index chlorophyll measurements

The ‘greenness’ of the leaves represents the amount of chlorophyll found in the chloroplasts. Leaf chlorophyll content can be used as an N status indicator, because this is an essential element in photosynthetic protein synthesis. Leaf chlorophyll content increases with N supply and decreases when N is limiting. Recent studies (see literature review for this project) have tested optical sensors to estimate N status in a variety of crops. The use of optical sensors

allows the assessment of a large number of plants in a short time period, and the results are immediately available.

The producers of the atLEAF⁺ device claim that the readings are as accurate as SPAD readings. On their website, there is an atLEAF⁺ to SPAD conversion application. The atLEAF⁺ sensor measures chlorophyll content in a small part of a leaf and not the whole plant and it is difficult to collect readings on small leaves from plants such as conifers. It was found that an average reading could be taken by holding down the reading button and passing a whole leaf/needle area through the aperture. The device's aperture is approximately 4 mm wide and that can cause a variation of readings of +/- 5 units, depending on the position of the leaf

(closer to the top part of the aperture or to the lower part). Secondly, this space allows light to reach the sensor diode and interfere with the reading. The atLEAF⁺ sensor has an open space and no filters, which makes readings sensitive to high light exposure.

In this first year of trials we have focused on the responses of the equipment and have not established a direct relationship between N tissue levels and the equipment readings. In the case of buddleja, an atLEAF⁺ unit of 55 equated to tissue N levels of 3-5 %. Tissue N levels were consistent across the different CRF levels and the two sites. In the Buddleja and Chamaecyparis subjects, the N levels started high and fell during the growing season. In the case of Viburnum and Skimmia this was reversed, with N rising in the tissue over the season.

The calibration of the two chlorophyll detection devices to give a direct N level was not possible in this first year. Both pieces of equipment have recommendations as to N application rates for specific crops. It should be noted that in the case of the atLEAF⁺ system, an increasing reading relates to a rising N application level. In the case of the Green Index system, an increasing number indicates lower N application rates.

Data output was related to the tissue analysis of N. The data from the Greenmount site is shown in Appendix 8 Figures 54-57. The atLEAF⁺ data used were a mean of the three replicates. In Figure 15, the atLEAF⁺ results are shown as atLEAF⁺ units and the leaf tissue as percentage N. There was a significant correlation ($P < 0.05$) found between the atLEAF⁺ readings and the leaf tissue N in species *Chamaecyparis* and *Buddleja*. In regard to all four species, a very highly significant correlation ($P < 0.001$) was found between the atLEAF⁺ readings and the Greenindex app readings. In the species *Chamaecyparis* and *Buddleja*, the atLEAF⁺ readings and Horiba nitrate meter readings were also found to significantly correlate to analysis results ($P < 0.001$).

The tissue levels of N followed a similar pattern to the atLEAF⁺ readings as shown in Figure 15. This was true for the Buddleja and Viburnum at Greenmount, but less so at PCS. The lower 2 kg/m³ and 4 kg/m³ CRF levels gave more consistent readings than the upper levels of 6 kg/m³ and 8 kg/m³. The atLEAF⁺ data for PCS were less consistent than the Greenmount data and shows that we need to record the levels of light at the time we take the readings. When all readings and species are put together, the trend of the atLEAF⁺ results has a similar slope to the tissue analysis.

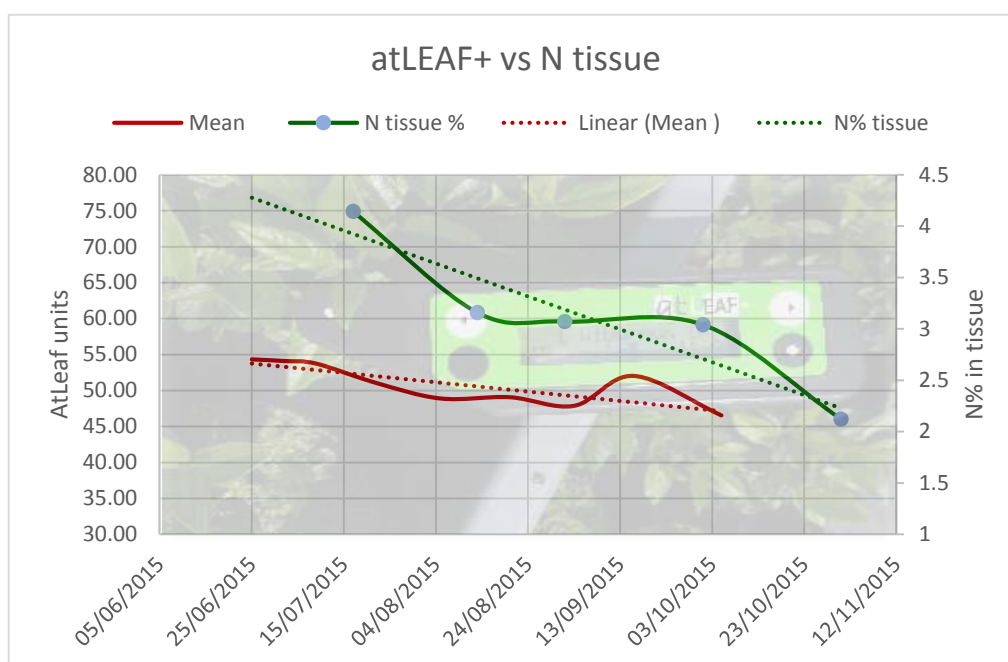


Figure 15. Illustration of atLEAF+ N values compared to leaf tissue N% by analysis
atLEAF+ chlorophyll measurements continued

The growing conditions in Belgium produced an entirely different set of data to Northern Ireland. Whilst the trend in Northern Ireland was for the N levels in the tissue to fall and then increase towards the end of the season, in Belgium they continued to fall throughout the period of the trial. Leaf colour however did not always follow these trends; a colour increase did not occur with the lower rates of CRF, but did with higher rates.

Buddleja

As expected, in *Buddleja*, as the CRF rates increased there was an improved correlation between the increasing levels of tissue N and the atLEAF+ readings (chlorophyll response). The greater the CRF application, the more N was utilised by the *Buddleja* plants.

Chamaecyparis

The technique for measuring the chlorophyll content of a coniferous subject required a series of readings to be taken as the needles passed under the sensor. This ensured a more representative sample of leaf was obtained. All readings from *Chamaecyparis* were taken this way. The conifer readings in Northern Ireland were inaccurate due to a wrong default setting on the equipment and have been discarded. The other crops at that site and at PCS gave useable data.

Skimmia

The *Skimmia* plants, whilst not in good condition did manage to grow in Northern Ireland and data were taken from the tissue and atLEAF+ device. No data was available from the plants at PCS due to death after potting. It was too late in the season to purchase replacement plants. We did however manage to obtain some early data sets from them, but the results were somewhat erratic, and are therefore not reported here.

Viburnum

Viburnum showed some correlation between the atLEAF+ and lab analysis data (see Figures 38 and 41 in Appendix 2 and 3), however it was again somewhat erratic between the four CRF treatment levels.

Greenindex App

The Greenindex app was delayed at PCS due to the late delivery of an iPhone so only three sets of readings were possible. The condition of the *Skimmia* did not allow many tissue samples to be taken.

Horiba 'LAQUAtwin' NO₃ N measurements

In this case, sap extraction from ornamentals is much harder than for the food crops this system is normally used on, demonstrated by the variable results in Figure 16. Quite large volumes of foliage material are needed. Freezing the foliage prior to squeezing improved the ease of sap collection. Results can be seen in more detail in Figures 62 - 67, in Appendix 10.

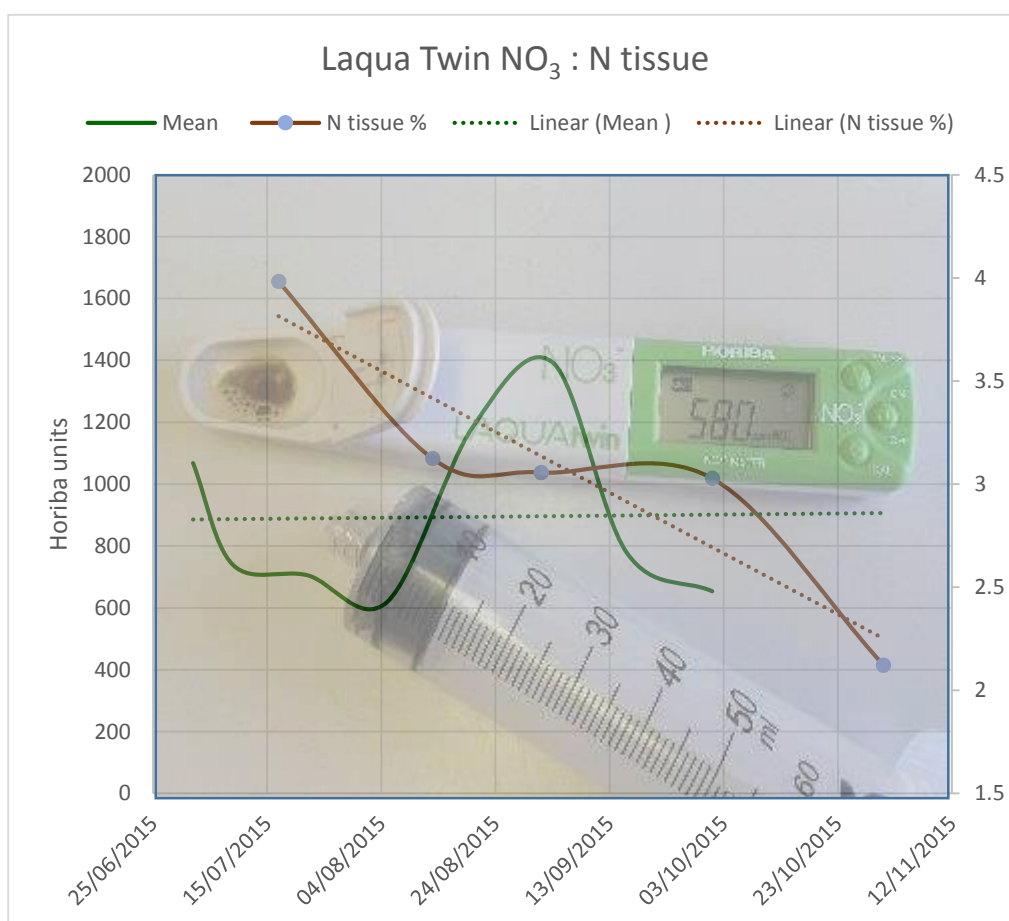


Figure 16. The relationship between N tissue measurements and the LaquaTwin readings – actual vs mean readings.

Decagon 'Procheck' with GS3 conductivity

This device gives an immediate display of substrate conductivity.

Figure 17 shows that substrate N level trends and Procheck data trends follow each other closely over the first year period of the trial.

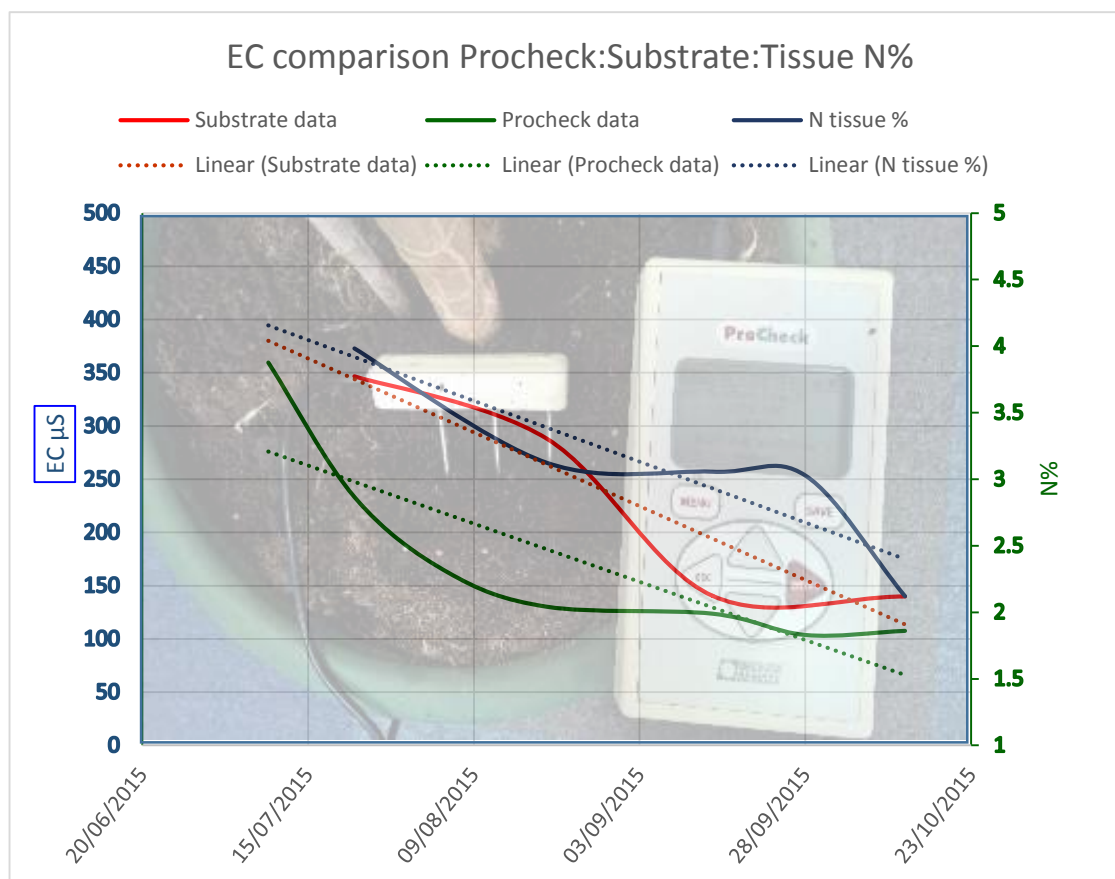


Figure 17. The relationship between the Procheck and the substrate analysis results over time.

Nitrogen leaching

Analysis of the rainfall and irrigation water collected by the 'pour-thru' devices provided data on the fate of the N applied to the substrate.

When we look at the total amount of nutrients leached from the Buddleja pots (per m²) we can see that plants treated with 8 kg/m³ Osmocote took up 4 times more N than plants treated with 2 kg/m³ Osmocote. The atLEAF⁺ and Greenindex both detected this difference but results were not clear.

The irrigation applications and rainfall at PCS are shown in the following chart, Figure 18

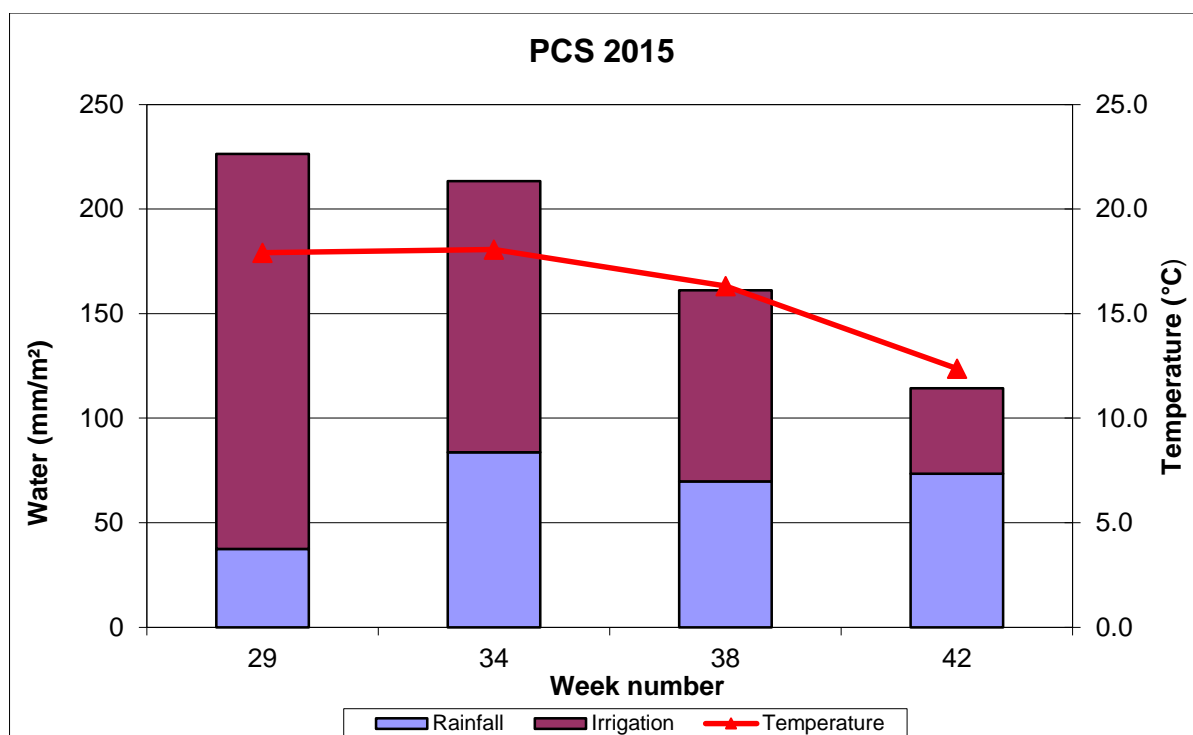


Figure 18. N levels in the 'pour-thru' water for the PCS Buddleja across time.

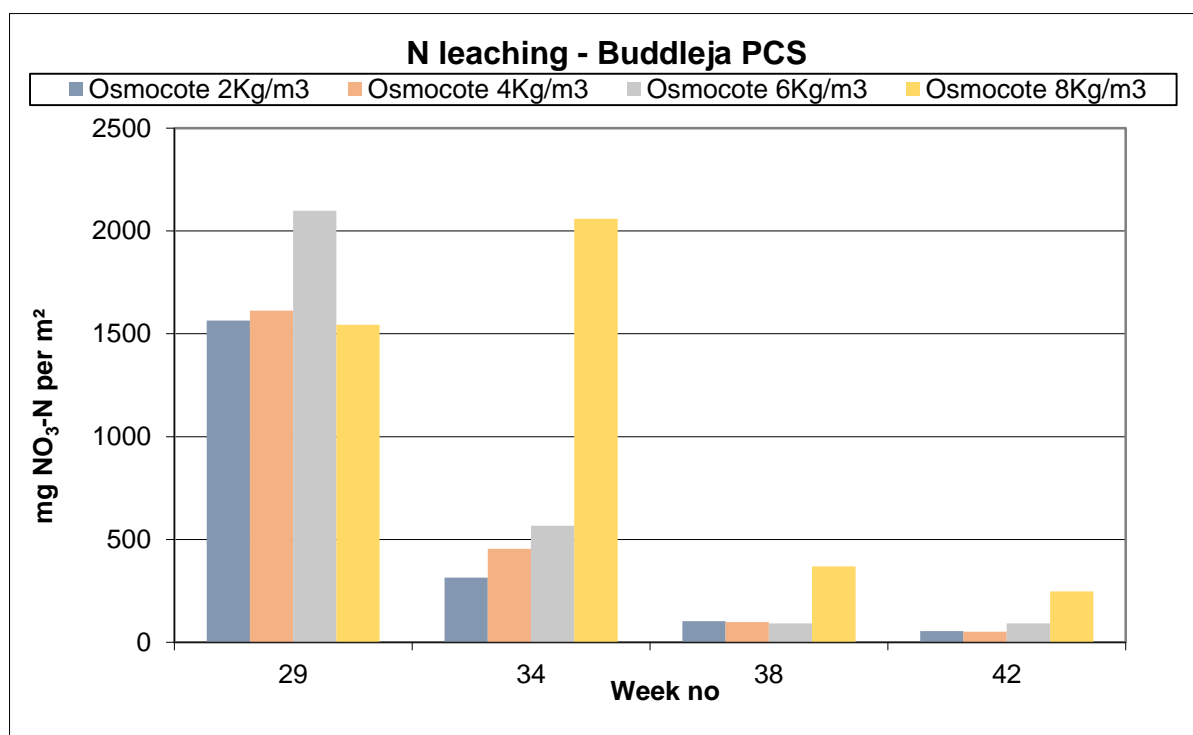


Figure 19. showing N leaching over time, and for each level of CRF in Buddleja.

The initially high levels of leached N seen in Figure 19 above are from the base fertiliser, CAN (calcium ammonium nitrate), used in Forest Gold® and some initial CRF release. We will have some real data on this in year two report.

Nitrogen balance in containers

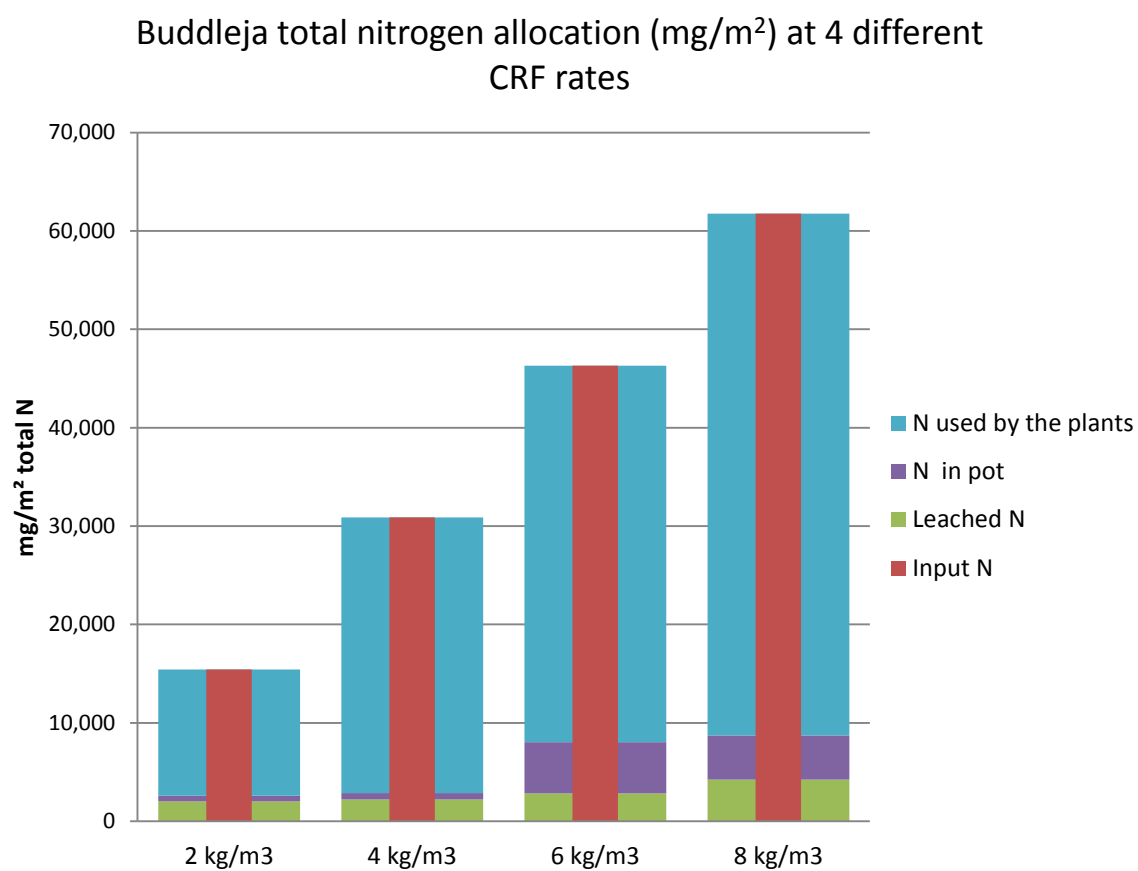
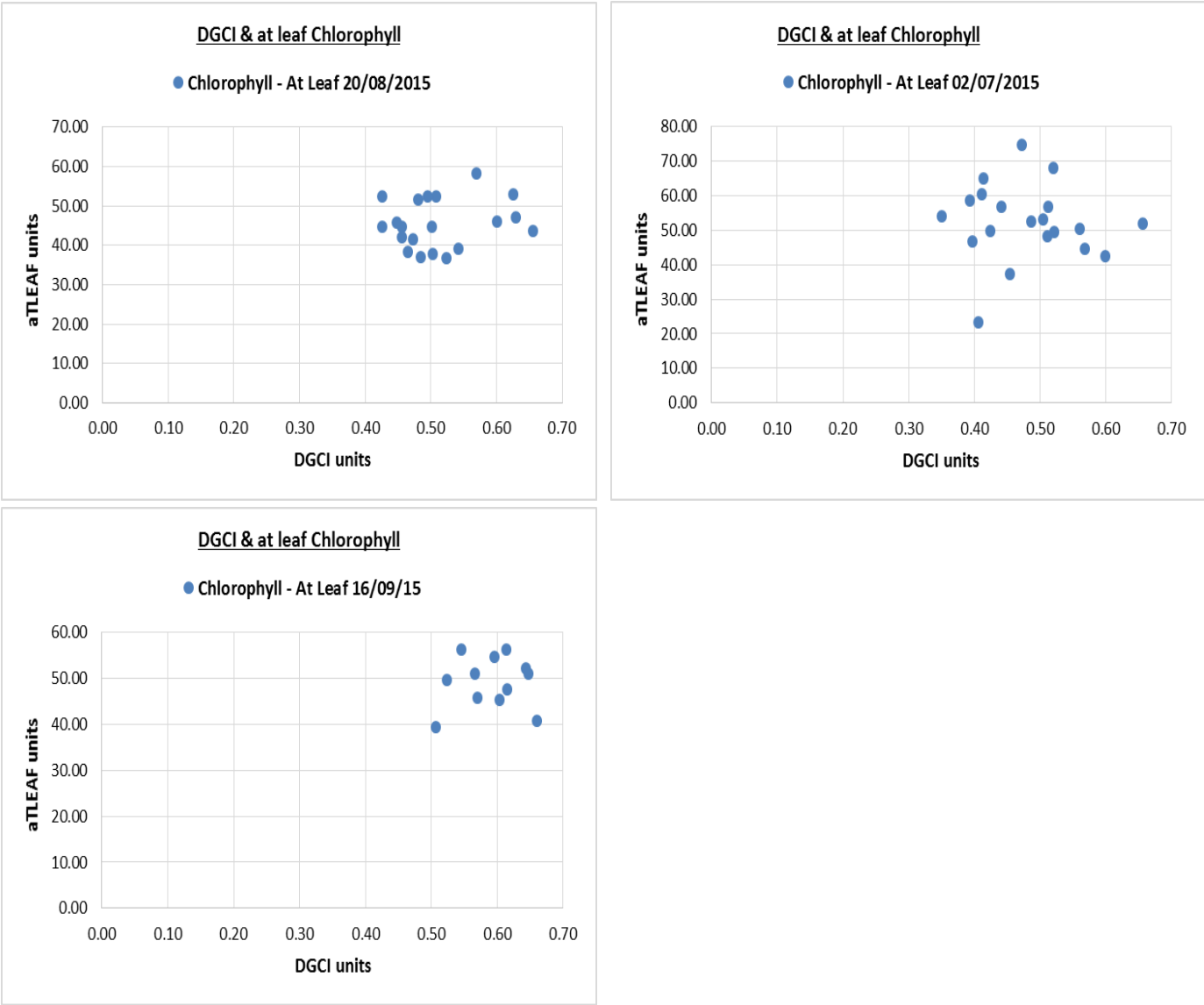


Figure 20. A limited amount of leaching loss and the apparent high capture of the N at all fertiliser input levels.

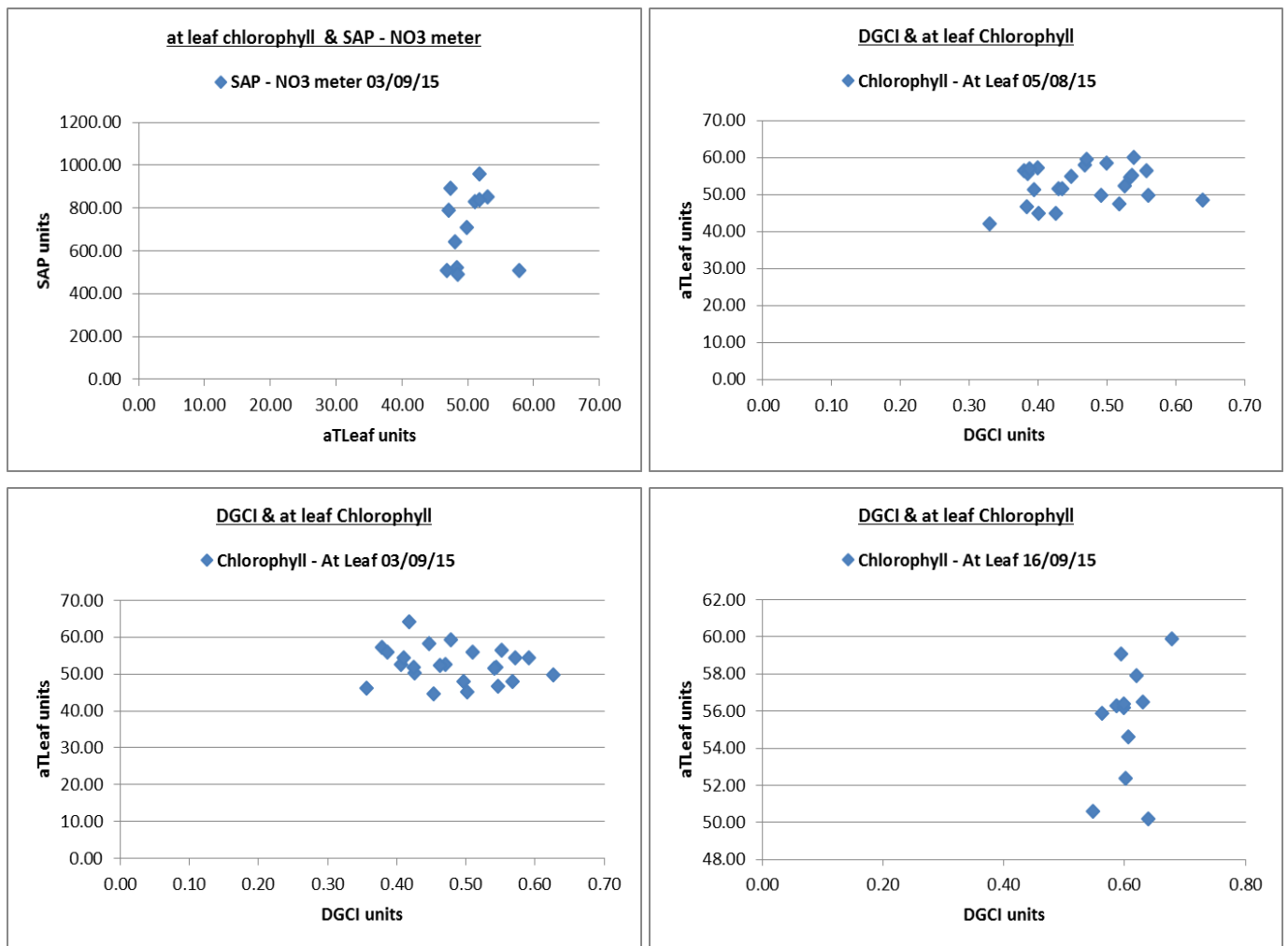
The data, graphically shown below demonstrates the statistical significance of equipment, and plant tissue analysis. Those of particular interest for specific plant subjects is shown below by way of example to the highly significant data sets in Appendix 11. The table in Appendix 12 shows the results of 62 of the 1531 variates that have a degree of significance.

Viburnum



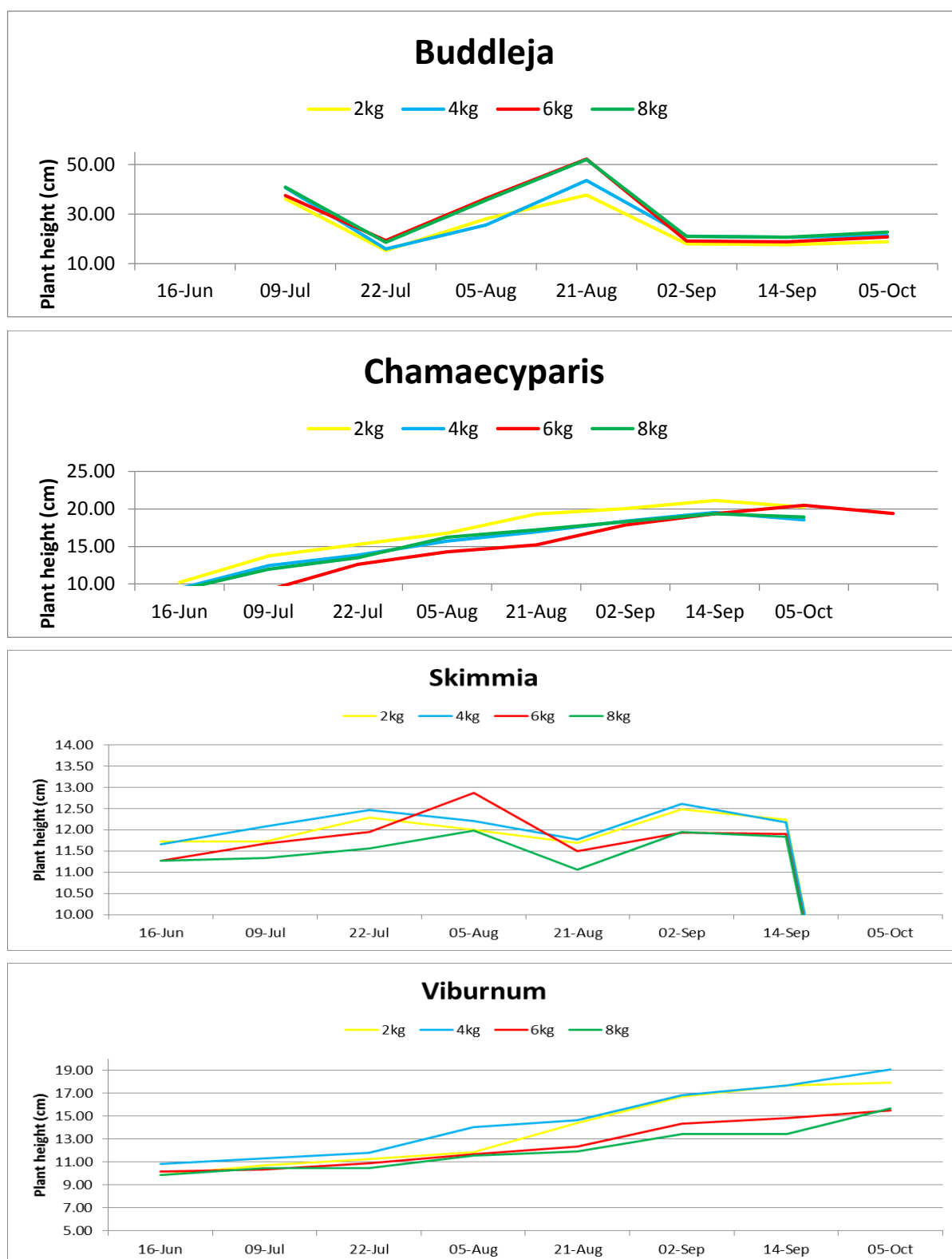
Figures 21, 22 and 23. Comparisons for Viburnum

Buddleja



Figures 24, 25, 26 and 27. Comparisons for *Buddleja*

Plant height



Figures 28, 29, 30 and 31. Illustrate height measurements made through the season.

Note the *Skimmia* results relate to very poor plants and therefore may not be a true reflection of growth. This relates to plant quality from the start of the trial. The peaks and troughs in the *Buddleja* relate to the trimming back incidences referred to in Materials and Methods section P 6. The *Viburnum* and *Chamaecyparis* gave the most consistent results, and illustrate a good relationship between height and CRF fertiliser application rates.

Discussion

The aim of this project is to examine the possibility for growers to make use of on-nursery tools/equipment to gauge the nutritional status of plants, without having to resort to destructive sampling and sending of samples to laboratories for analysis. The latter is seen as relatively expensive and is generally only done if there appears to be a problem with the growth of plants, by which time the results may show that recovery is almost impossible in any one growing season. For some years now growers have wanted the ability to constantly monitor crops and be able to make any nutritional adjustments to avoid the quality of the plants deteriorating beyond recovery.

Our tests include very simple 'dip stick' type assessment tool, through to the more sophisticated chlorophyll meters which assess the fluorescence of chlorophyll. Growers can use optical sensors to monitor plant N levels using sensor readings to make real-time decisions on the need for additional fertilisation. Growers can then produce a higher quality product, saving money on fertilisers and reduce pollution in the environment.

Challenges in this type of work include samples from the 'marker' crops on the nursery having to be collected at similar times of the day and from a similar age of plant material. In order to achieve this it is necessary to have someone on the nursery who is responsible for sampling and brings together all the results so that any emerging trends can be assessed.

Eventually it should be possible to see if the nursery results can be used ahead of visual symptoms appearing, in order to correct any nutrient shortfalls.

Collecting both rainfall and irrigation inputs and run-off outputs will allow some simple estimates of the nutrient use efficiency savings that could be made, and particularly the potential for reducing diffuse pollution from the fertiliser combinations used. In addition to the work described in this report, a 'bench scale' leaching trial is being run with a view to assessing which fertiliser components are major contributors to potential nutrient losses from the system. Work at RHS Wisley will also complement our ongoing work and broaden our information by looking at a wider range of substrate mixes.

Conclusions

In spite of the teething problems with plant material and learning to use the equipment some worthwhile results have emerged from the mass of data collected, however these results from the first year of the project should not be seen as definitive.

1. Equipment can be used to measure the nutritional status of a small range of ornamental crops, however these crops represent a range of leaf size and nutritional requirements, highlighting the breadth of what can be monitored.
2. The Green Index app needs further testing to confidently establish a link between N and app readings.
3. Horiba LAQUA twin is difficult to use on the nursery. A better sap extraction method needs to be established and will be explored further during the second year of work.

4. The atLEAF+ device is very promising, with readings showing a good relationship to N tissue levels.
5. Procheck & GS3 show a good correlation between N tissue levels and substrate EC.
6. The highest N leaching rate takes place in the first 4 weeks of cultivation (this is normal), and after week 34 there is almost no N leaching out from the pots. Leached quantities for 8 kg/m³ CRF is higher in Buddleja than the other treatments, suggesting that 8 kg/m³ might be more than needed to sustain the growth of Buddleja.
7. Plant trimming increased nutrient pour-thru, with higher N levels in the pour-thru water.

Actions from the results and conclusions from the year one work, to be included in year two are:

1. We need to monitor the ambient light levels for 4 hours prior to taking the chlorophyll readings, to ensure that the results are not adversely skewed due to large variations in the incident light levels.
2. We have identified some research that looks at Refractometer readings of plant sap as means of measuring N levels. We will be using a new piece of equipment to investigate this.
3. Due to the difficulties with sap extraction we will be using a new published method of sap extraction.

We also need to look again at the sap testing in relation to cleaning the solution by the use of active charcoal. This will achieve a cleaner sap sample that is free from plant tissue colouring which make readings difficult in the Merck strips and LAQUAtwin Nitrate device.

Knowledge and Technology Transfer

Over the coming season three open days are planned at the three trial sites. The PCS site will be a two-day event on 6-7th July 2016 and will include visits to Belgian ornamental nurseries.

One article has been written for the AHDB Grower magazine and another article is planned in the autumn of 2016. Two presentations have been made to growers during year one.

Two nutrition workshops are planned in December. The first on the 7th at the HTA offices in Oxford and the second on the 8th at STC in York.

References

- Smith D.L. (1987). Plant sap analysis as a monitoring technique for tomatoes in Rockwool. Stats of Guernsey Annual report 1986
- Le Marchant-Smith D. (1995). Monitoring the nutrition of clematis using plant sap analysis. Acta Hort 401 Growing Media & Plant nutrition. P 389-392.

Appendices

Appendix 1 - Weather data – Figure 32 – 35

The two sites each collect weather data on a half hourly basis. The data recorded for the trial purposes are average daily air temperature, rainfall and W/m². The comparisons between the two sites is shown in the following four charts.

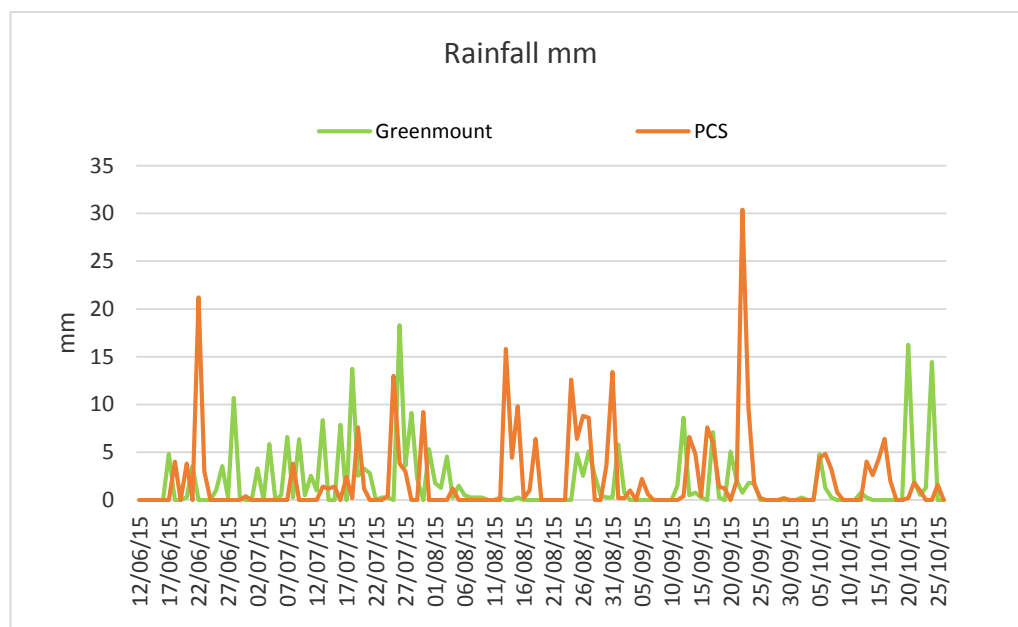


Figure 32. Average rainfall comparison between PCS and Greenmount

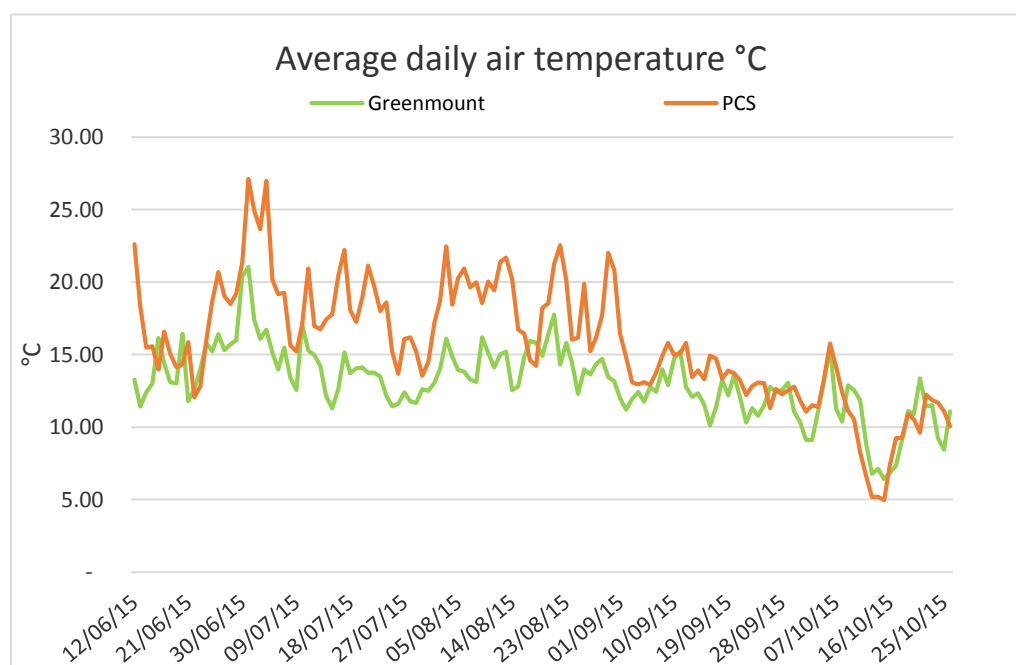


Figure 33. Average daily air temperature comparison between PCS and Greenmount

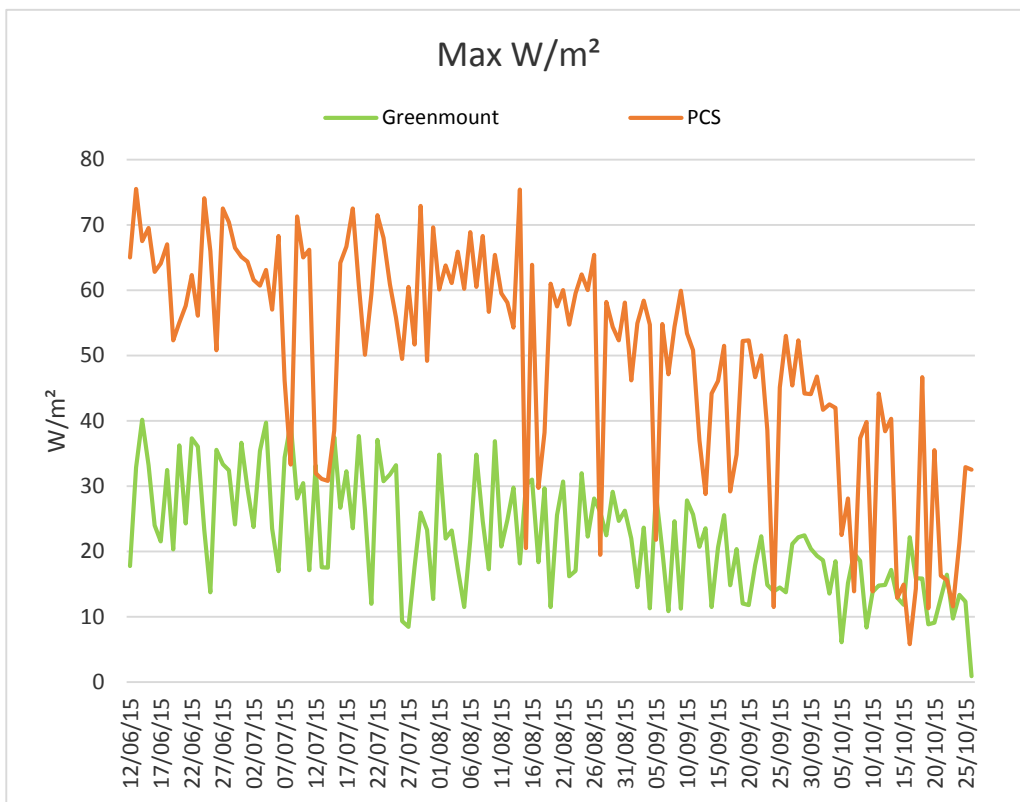


Figure 34. Average maximum W/m^2 comparison between PCS and Greenmount

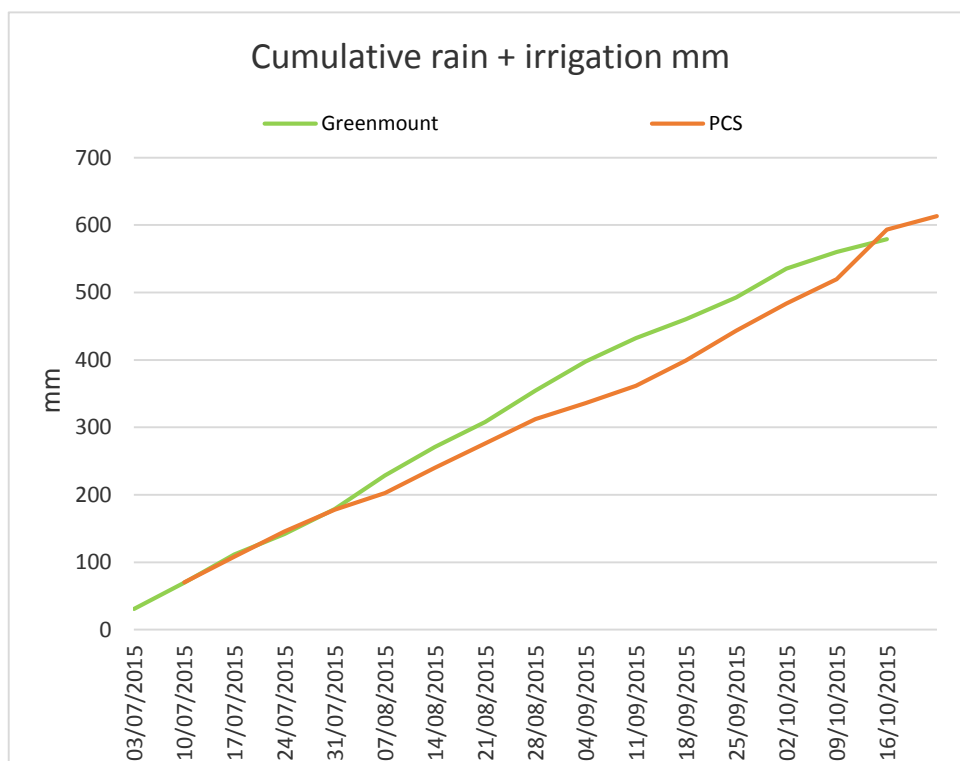
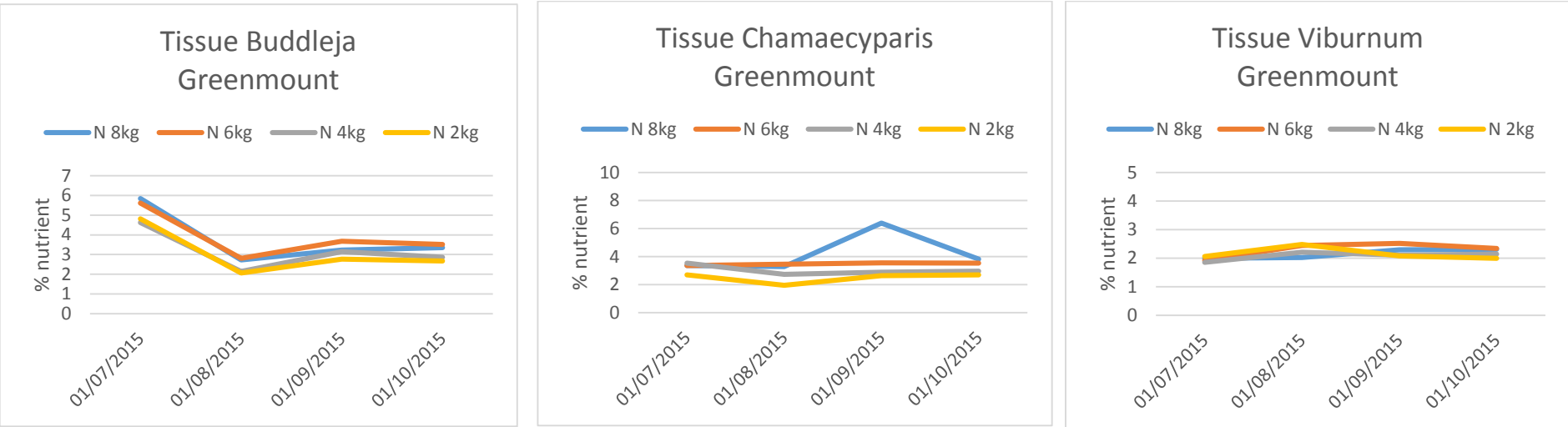
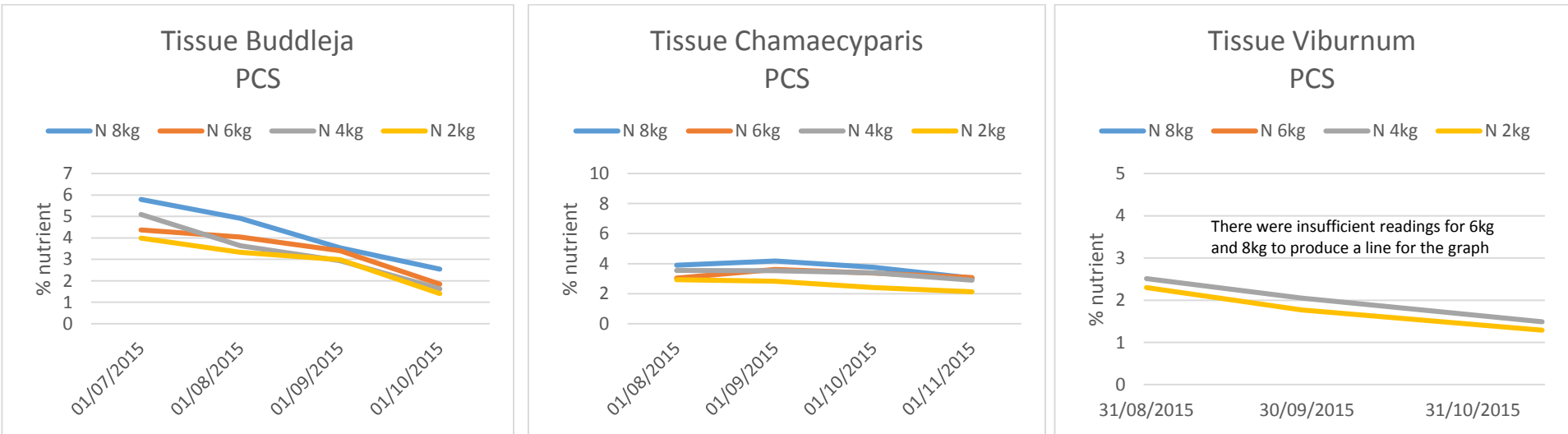


Figure 35. Cumulative rain and irrigation comparison between PCS and Greenmount

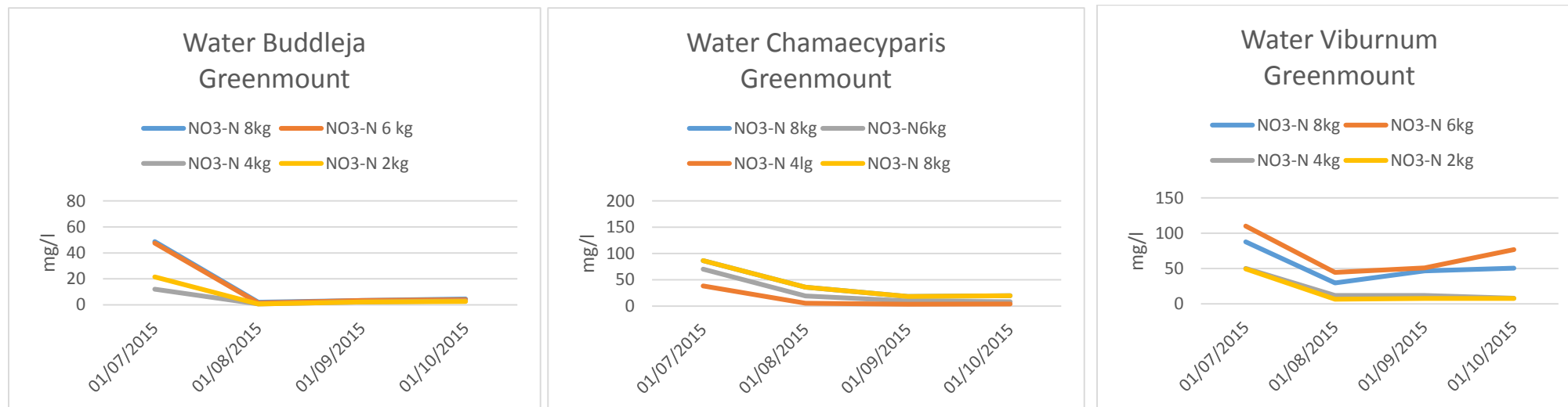
Appendix 2 – Tissue Data – Greenmount Figure 36 - 38



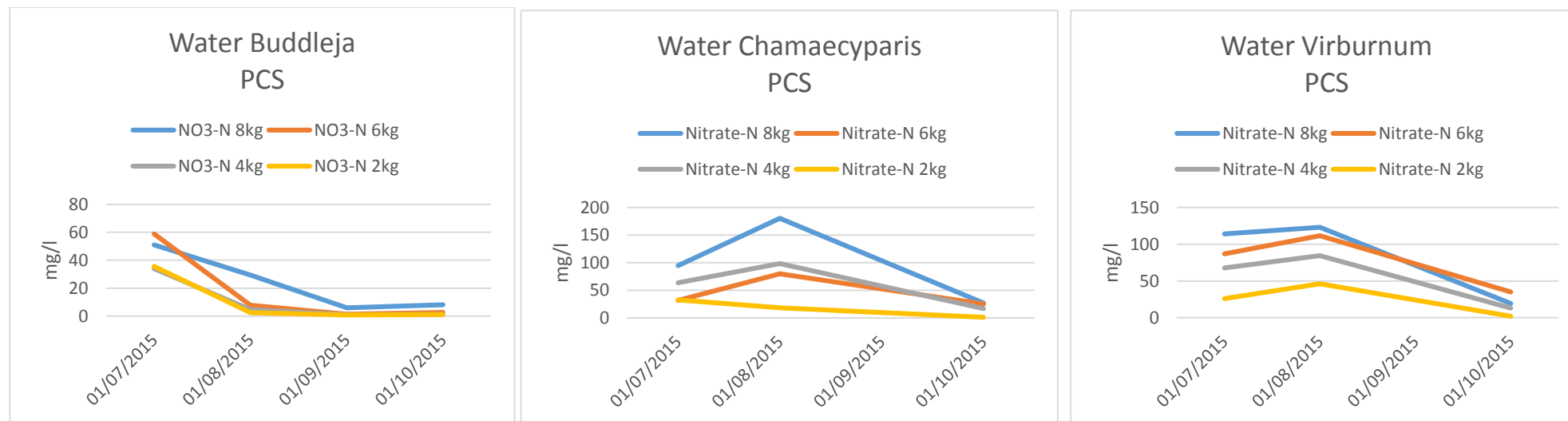
Appendix 3 – Tissue Data - PCS Figure 39 – 41



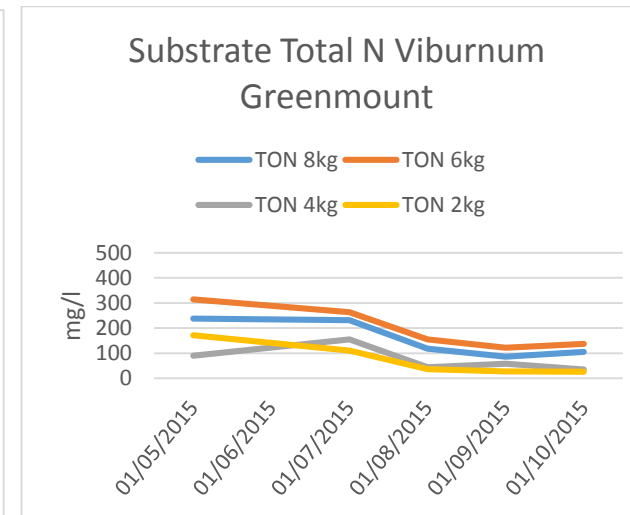
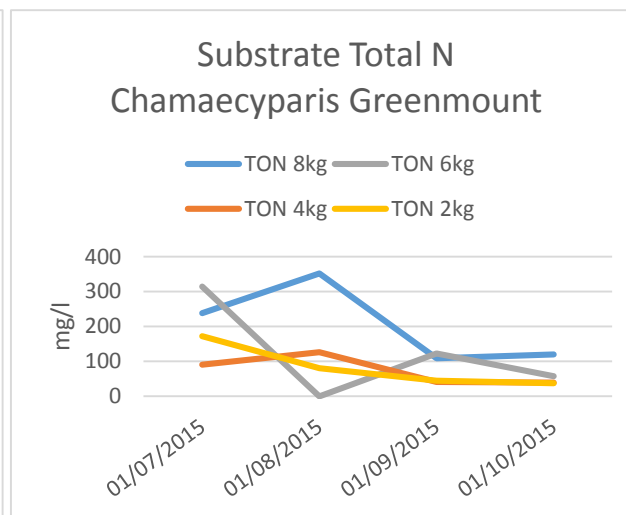
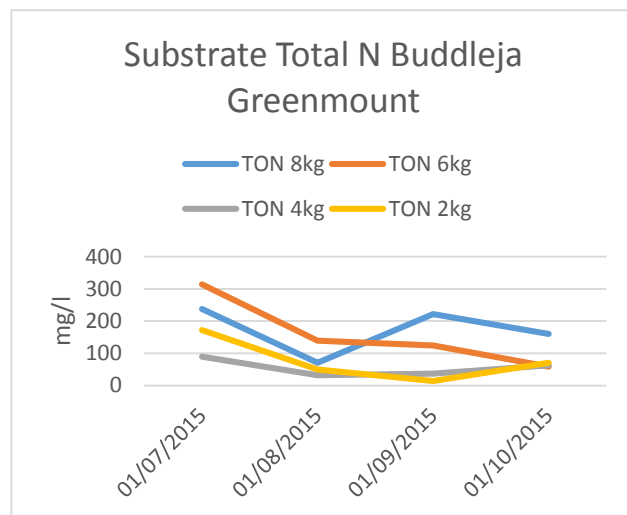
Appendix 4 – Water Data - Greenmount Figure 42 – 44



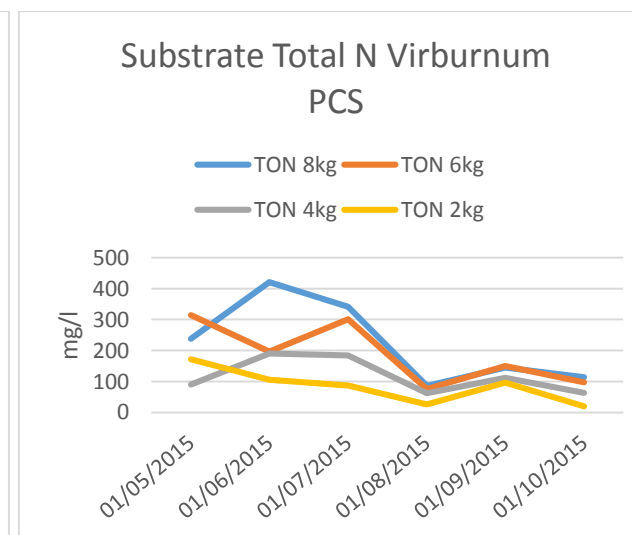
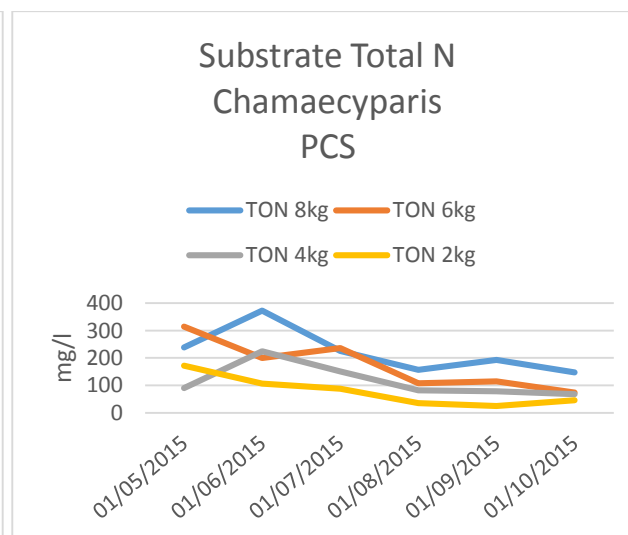
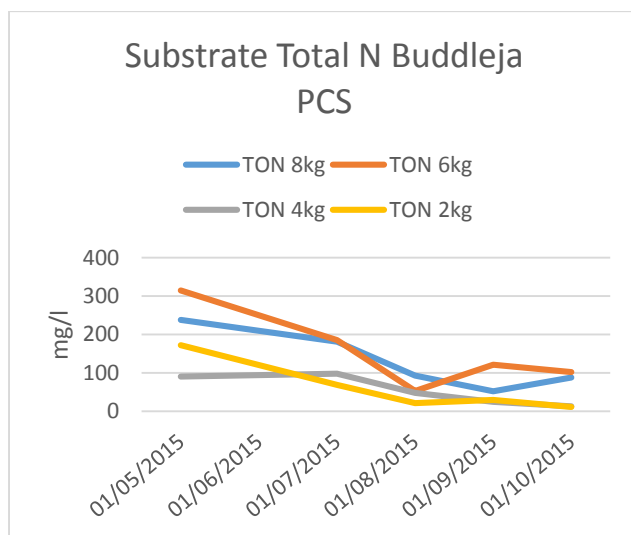
Appendix 5 – Water Data – PCS Figure 45 - 47



Appendix 6 – Substrate Data - Greenmount Figure 48 - 50



Appendix 7 – Substrate Data – PCS Figure 51 – 53



Appendix 8 – Tissue atLEAF⁺ Figure 54 - 57

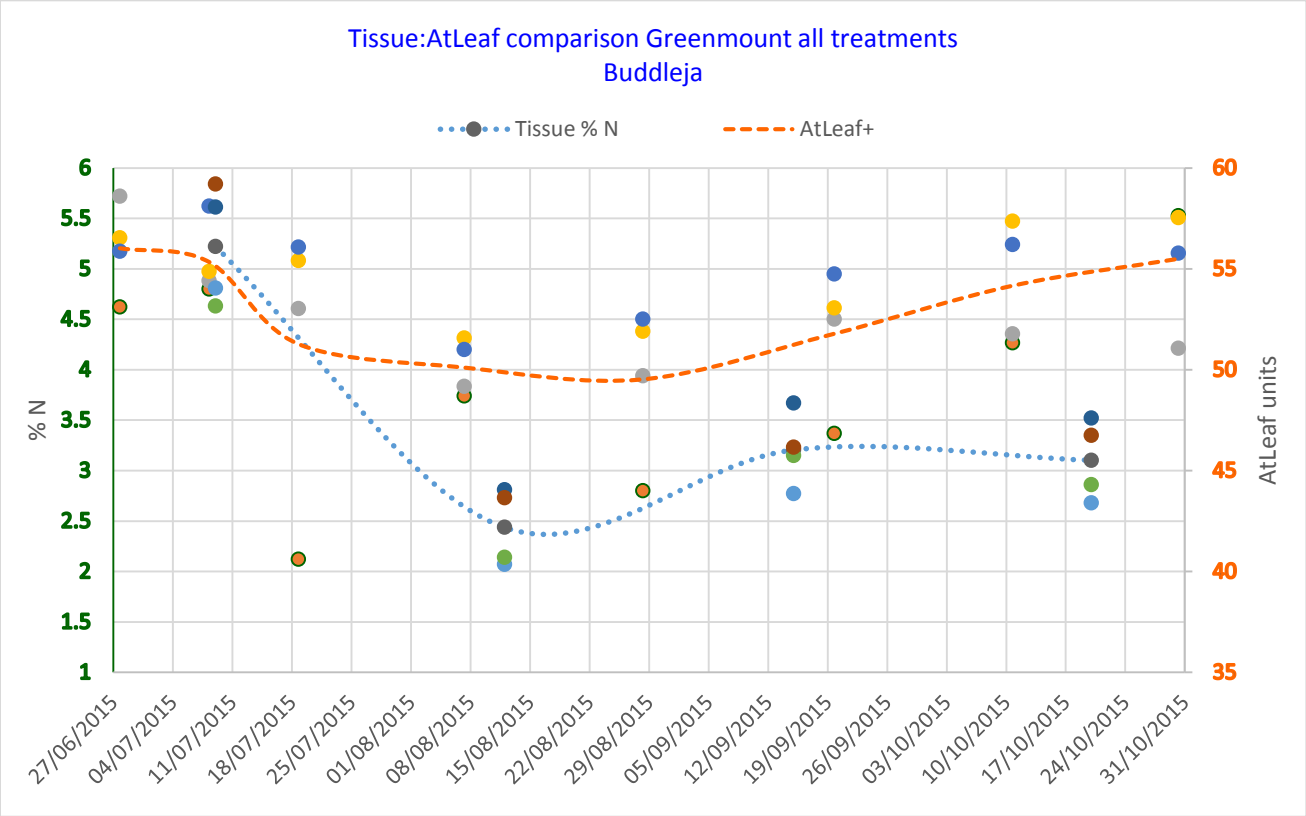


Figure 54

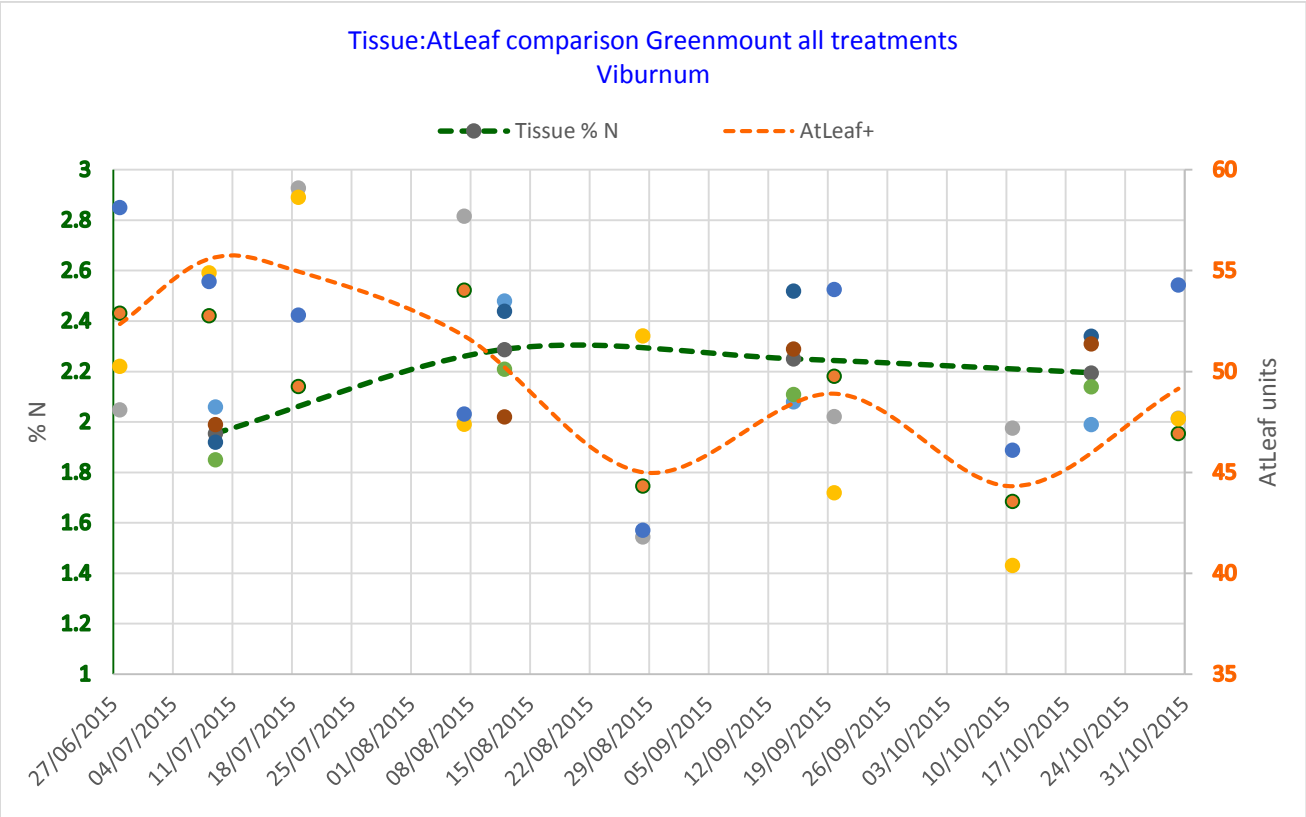


Figure 55

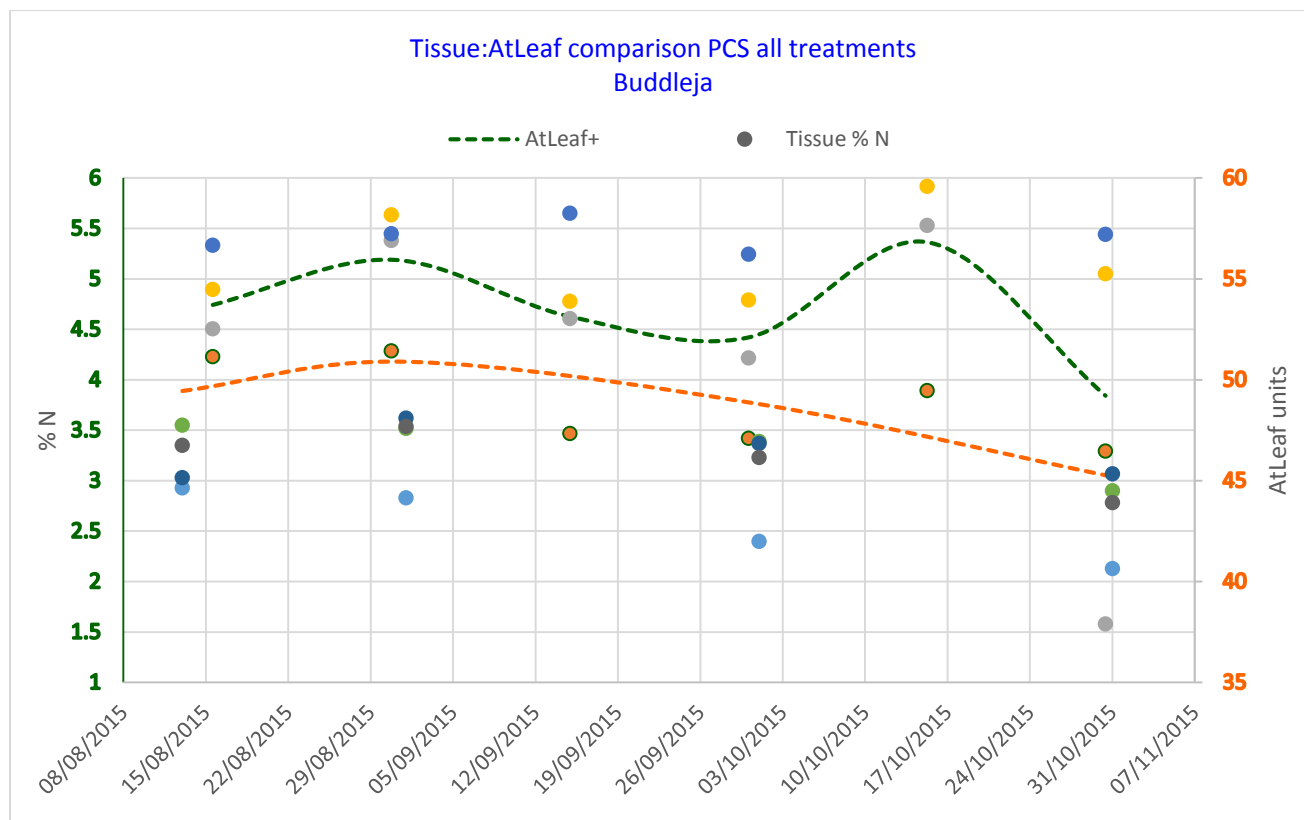


Figure 56

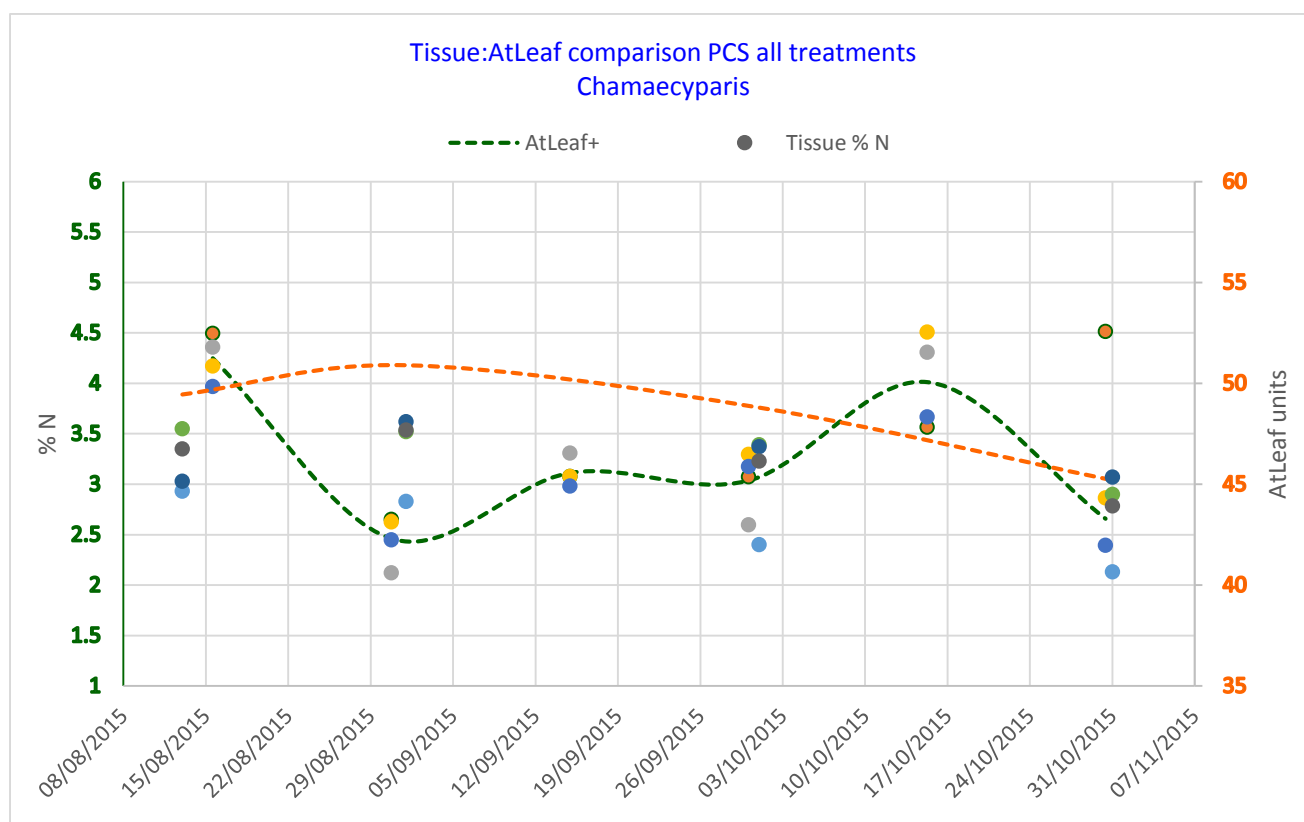


Figure 57

Appendix 9 - Tissue ‘Greenindex’ Figure 58 - 61

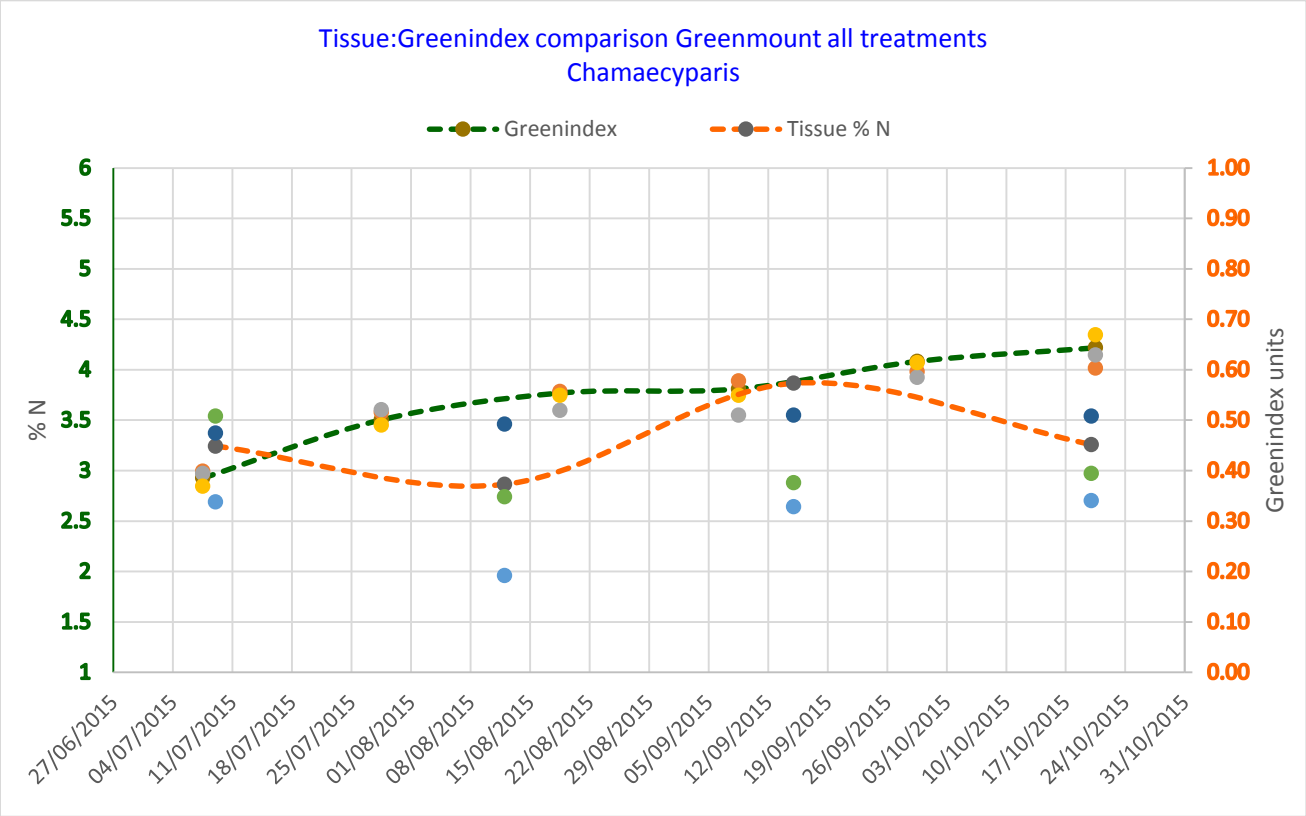


Figure 58

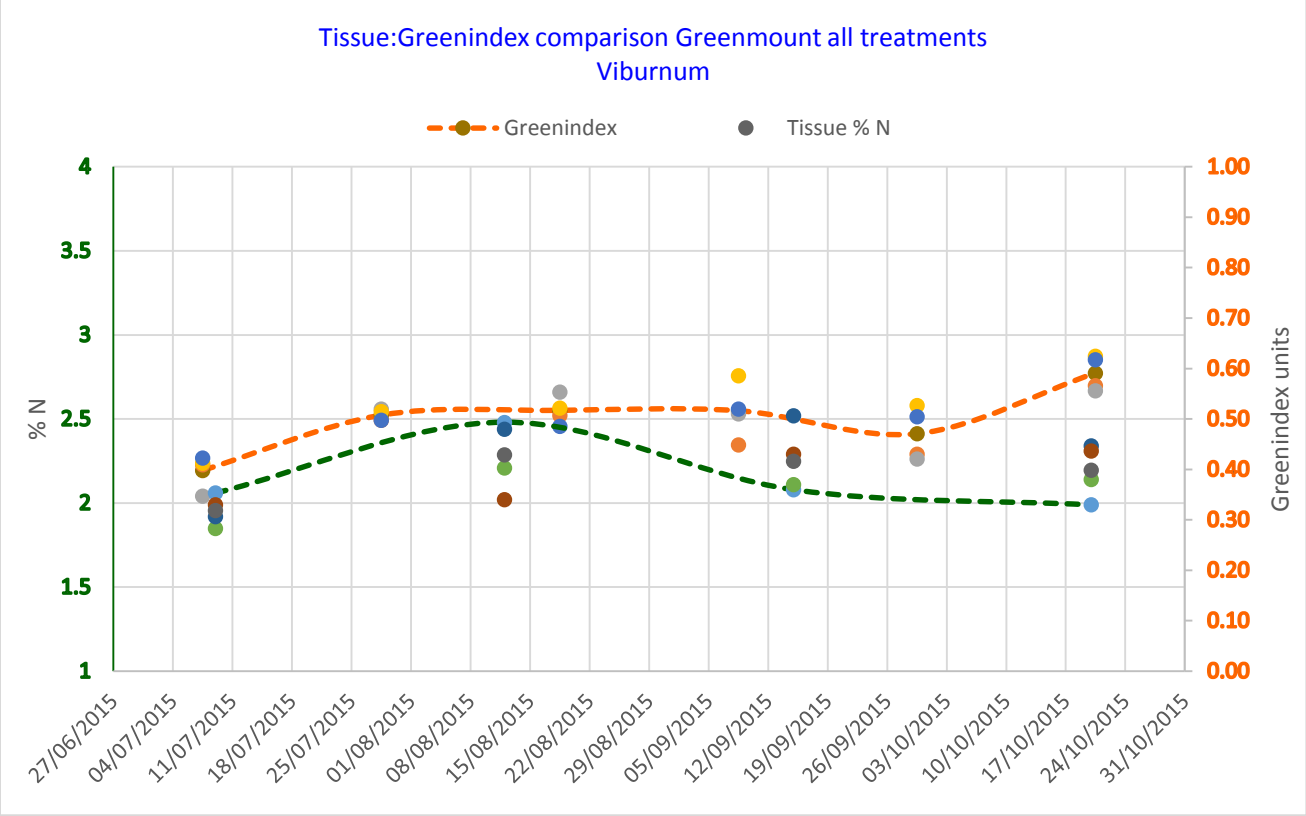


Figure 59

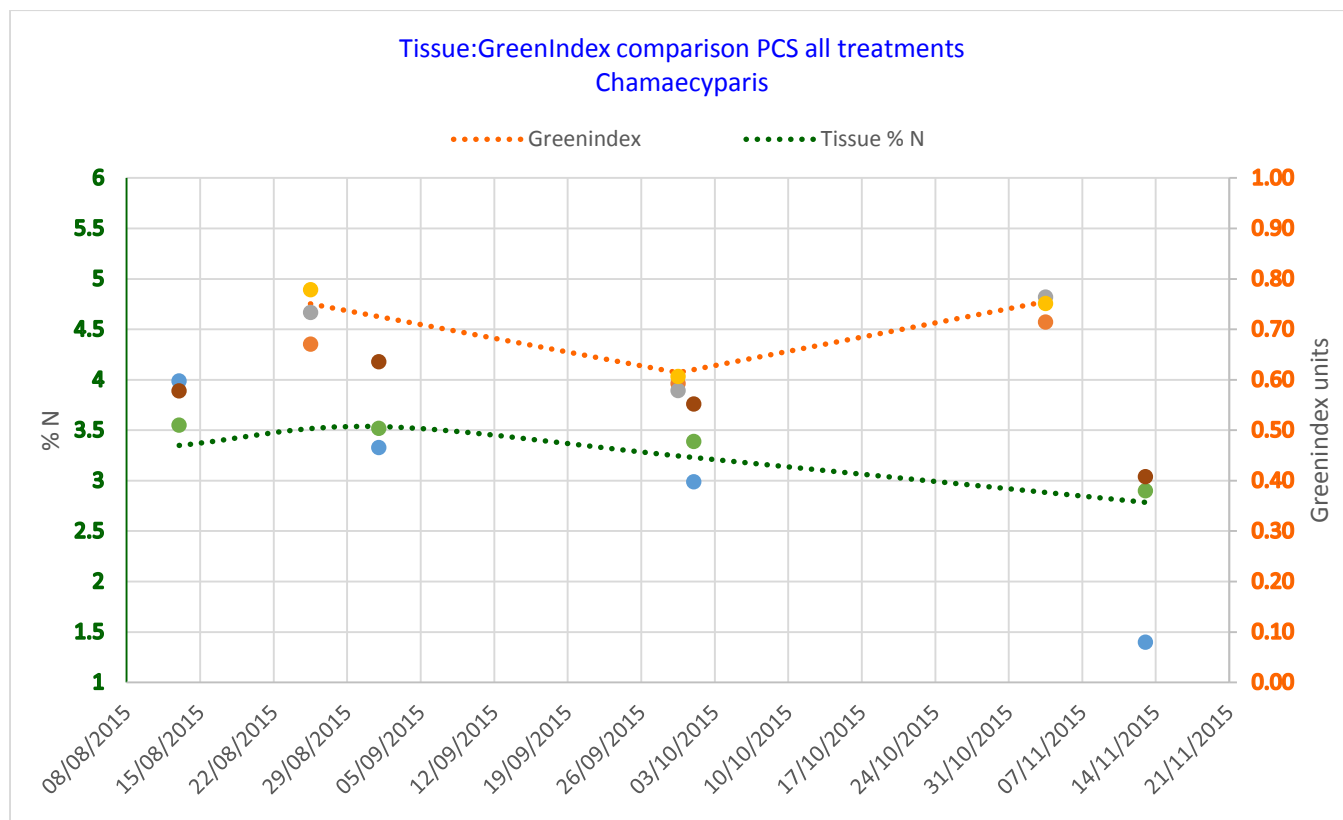


Figure 60

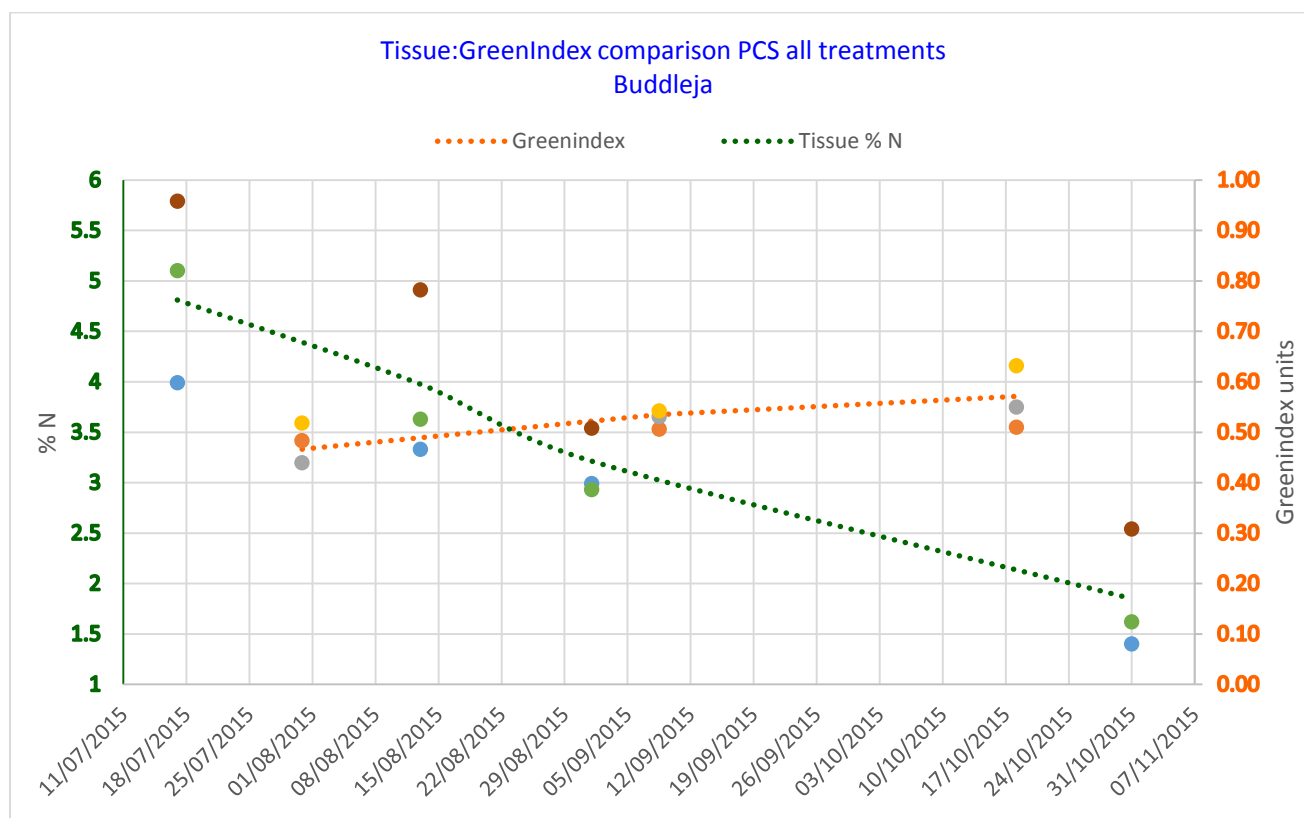


Figure 61

Appendix 10 - Sap tissue 'Horiba LAQUAtwin' Figure 62 – 67

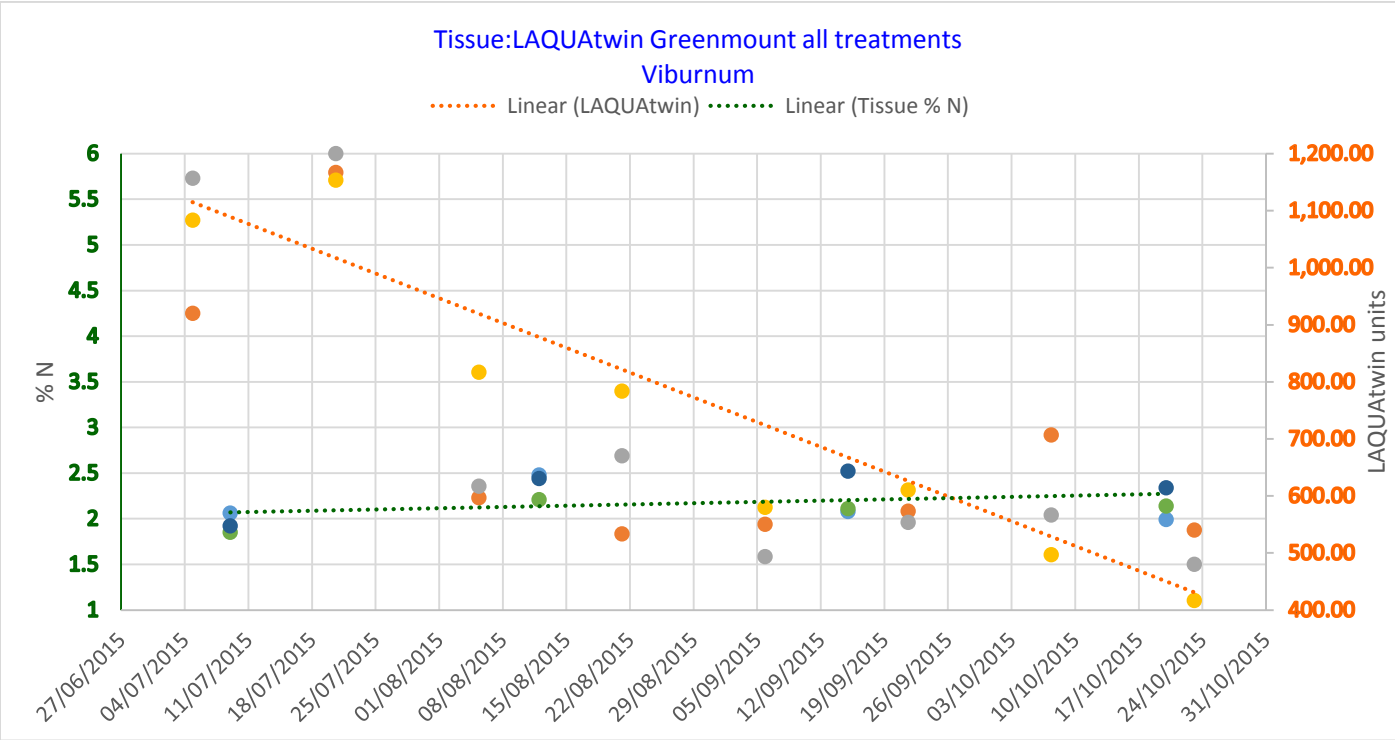


Figure 62

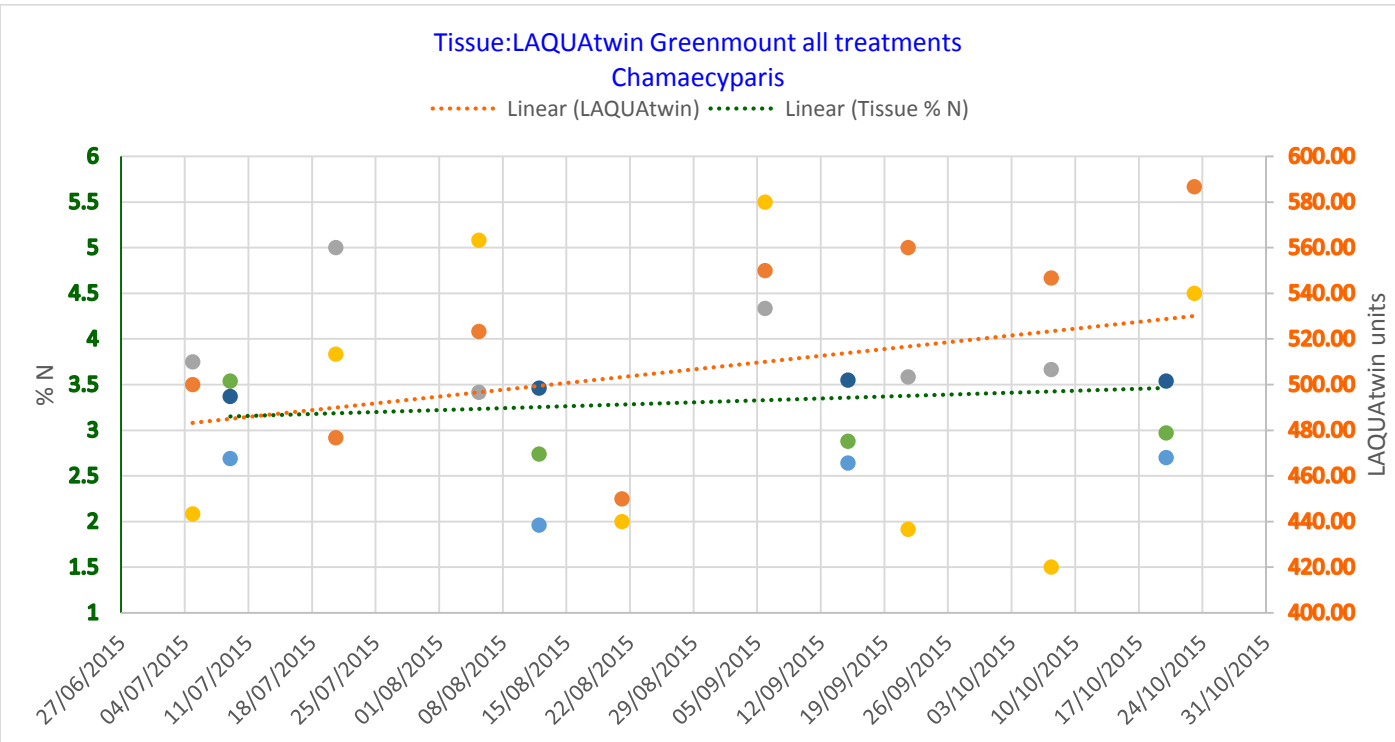


Figure 63

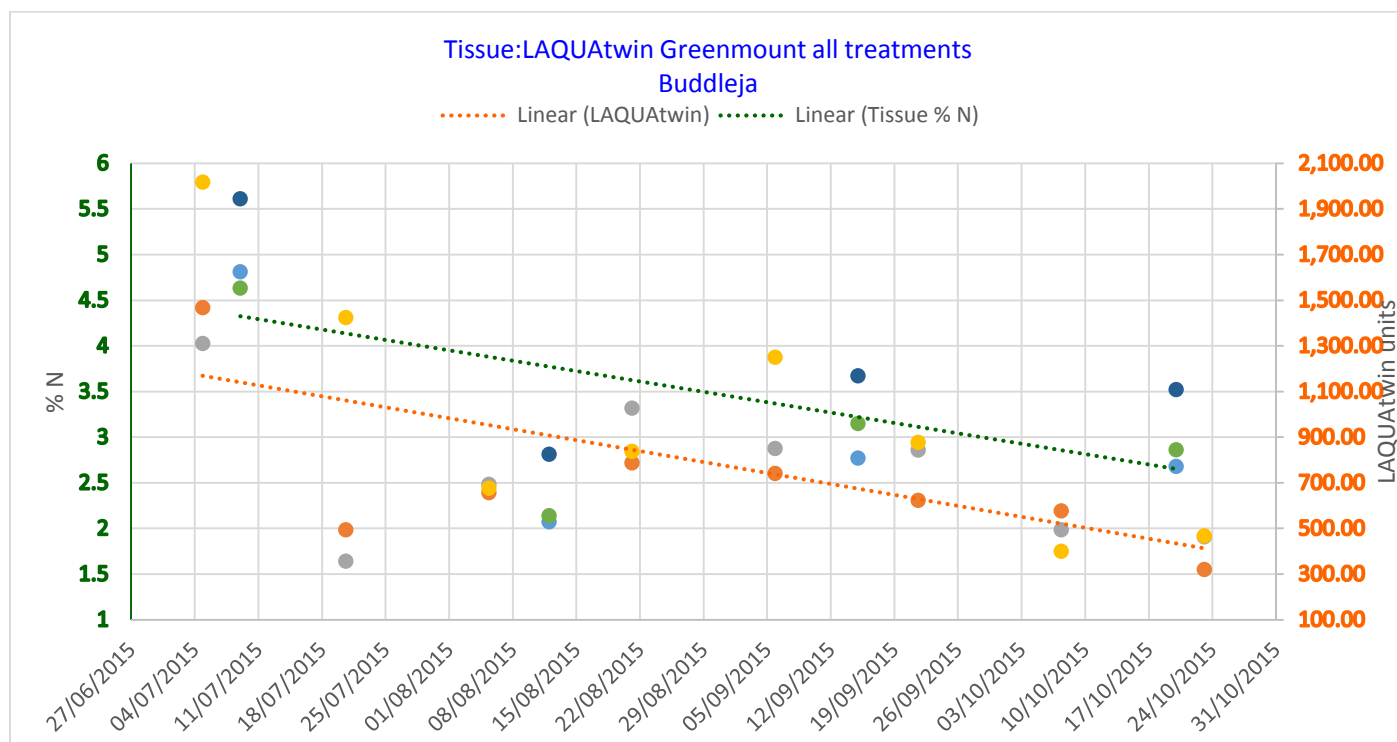


Figure 64

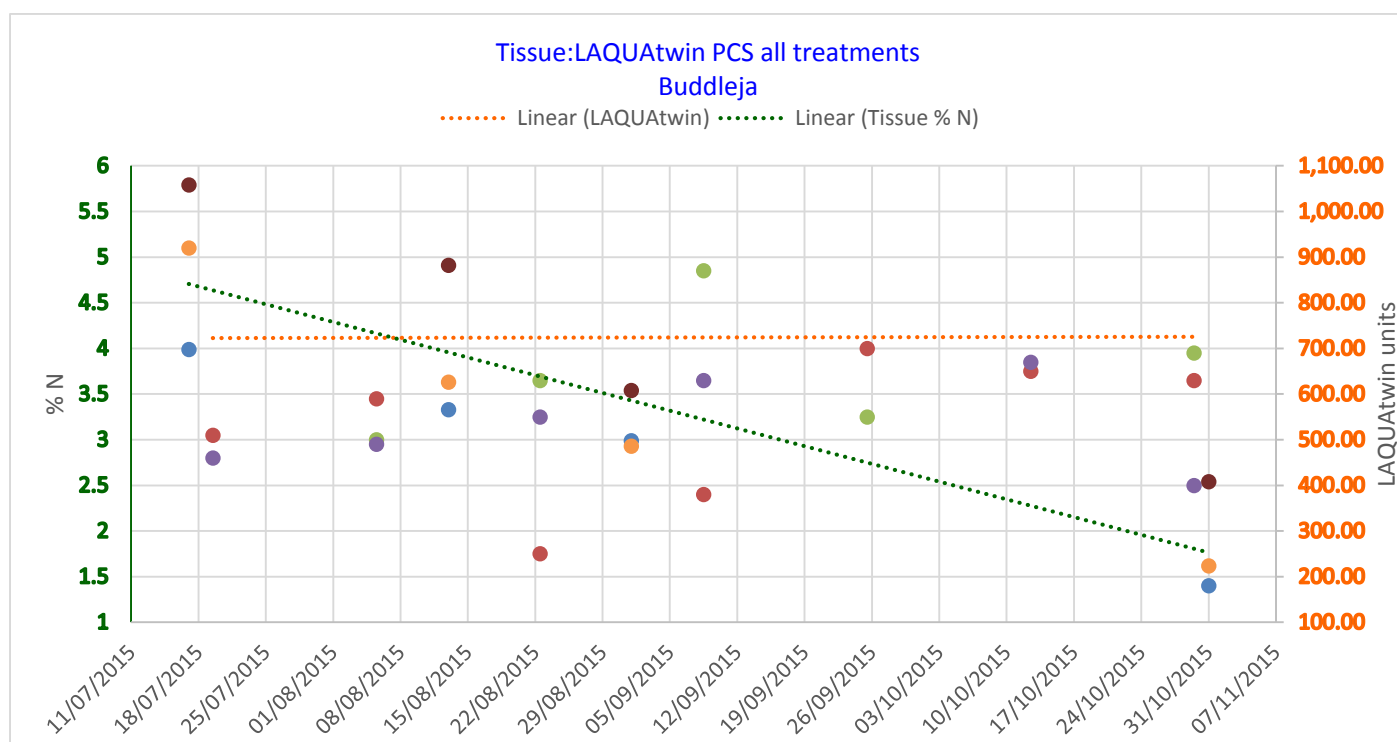


Figure 65

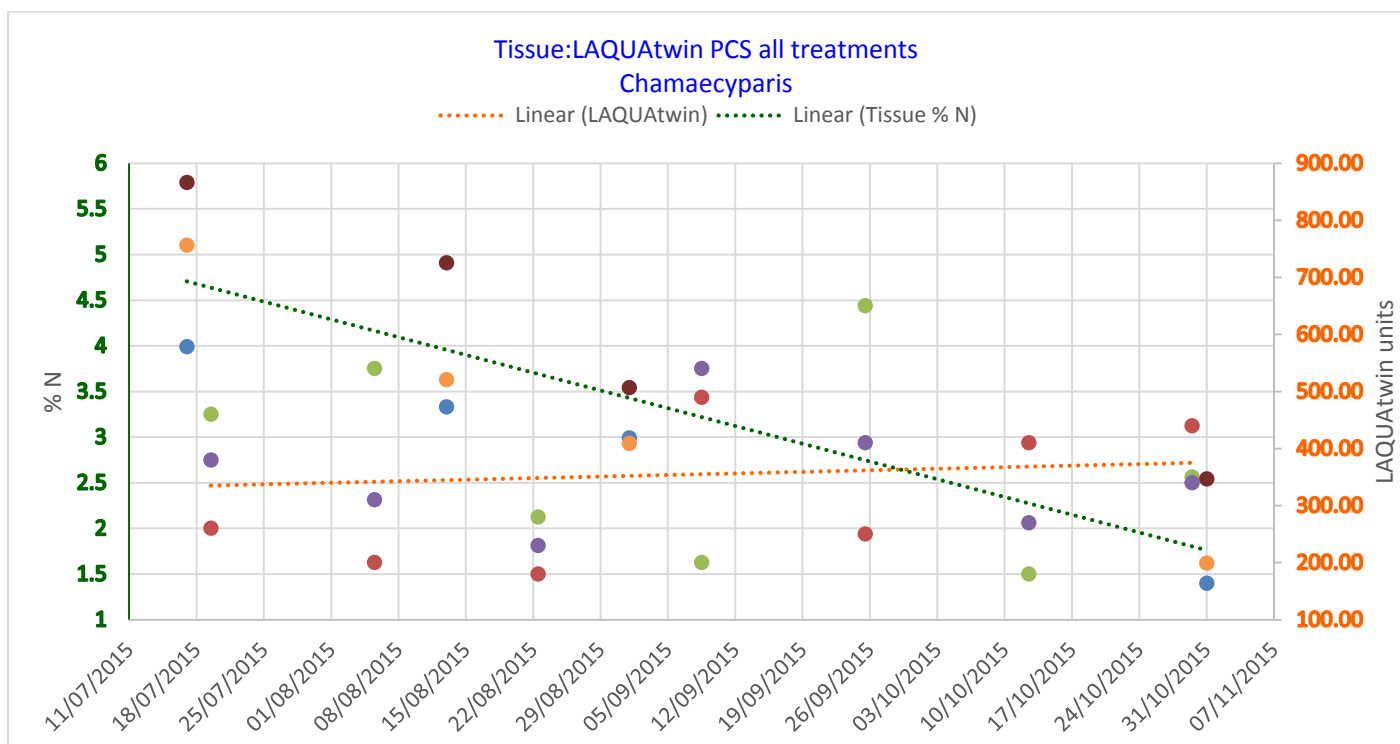


Figure 66

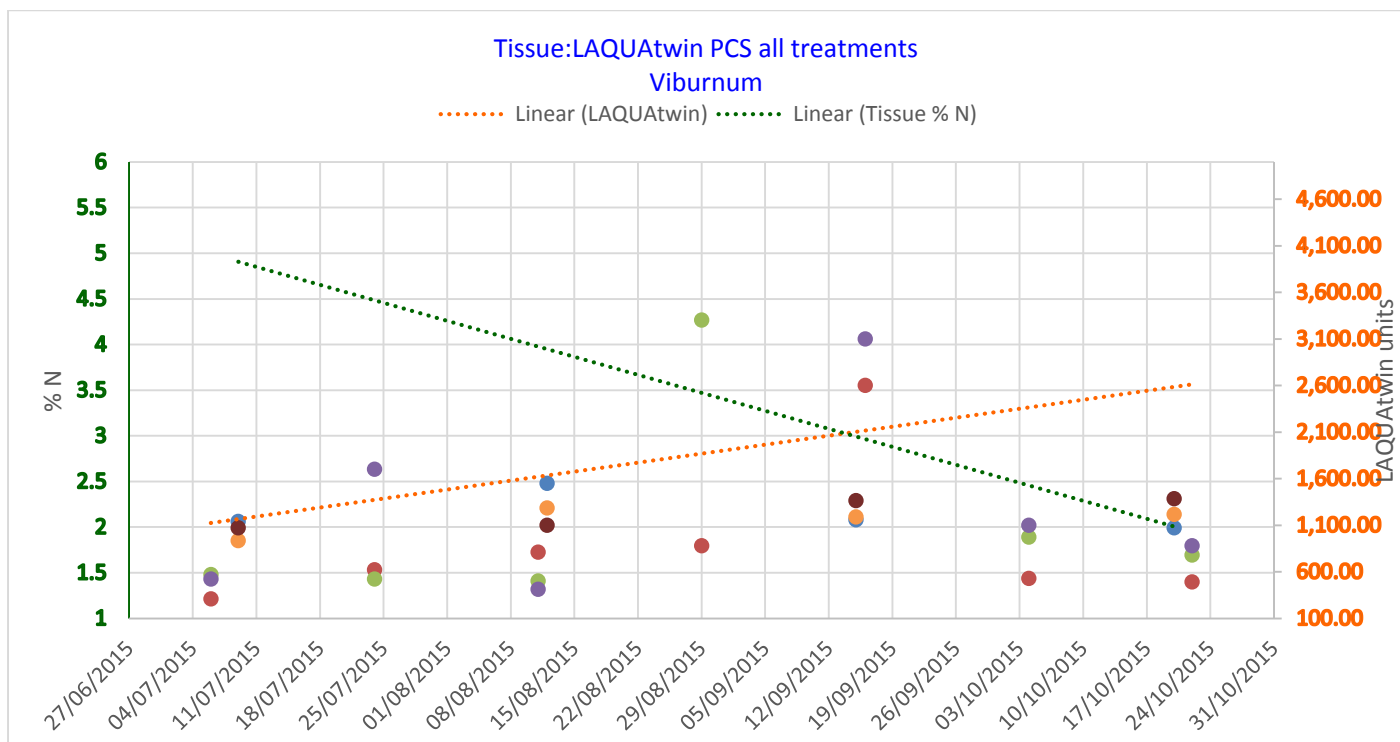


Figure 67

Appendix 11 – Statistical Analysis of Results - graphs

Viburnum

AT Leaf Chlorophyll & Heights (Figure 68)

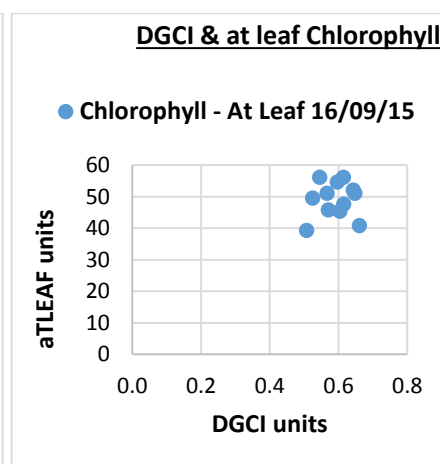
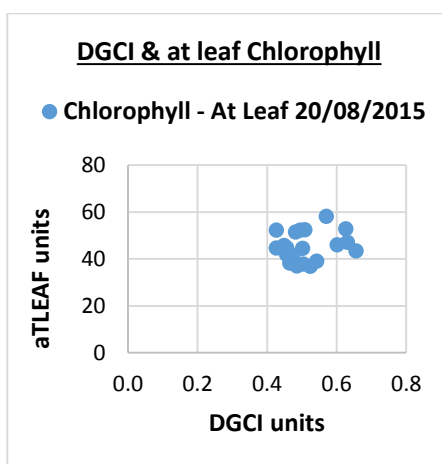
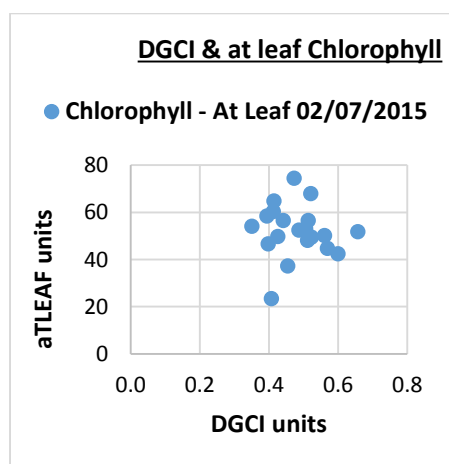
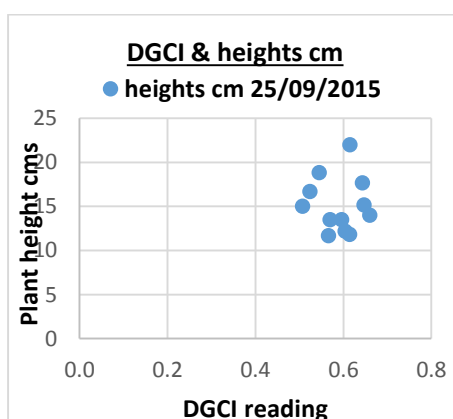
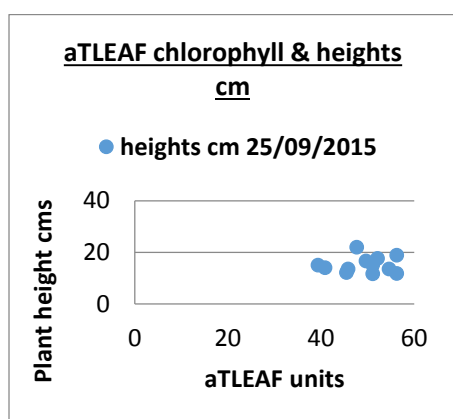
Correlation was found between the AT leaf chlorophyll meter and the height of plants mid season. As expected as the plants produced new growth chlorophyll levels were found to correspond with growth.

DGCI & Heights (Figure 69)

As found with the AT leaf chlorophyll meter in Figure 68 the Green index app DGCI was found to correlate with plant height in mid season. The DGCI and measure of dark green colour index was found to correspond with plant growth.

DGCI & AT Leaf Chlorophyll (Figures 70-72)

The Green index app, DGCI measure of dark green colour index and the chlorophyll measure from the AT Leaf chlorophyll meter was found to correlate for the Viburnum plant during both the early and mid season growth period.

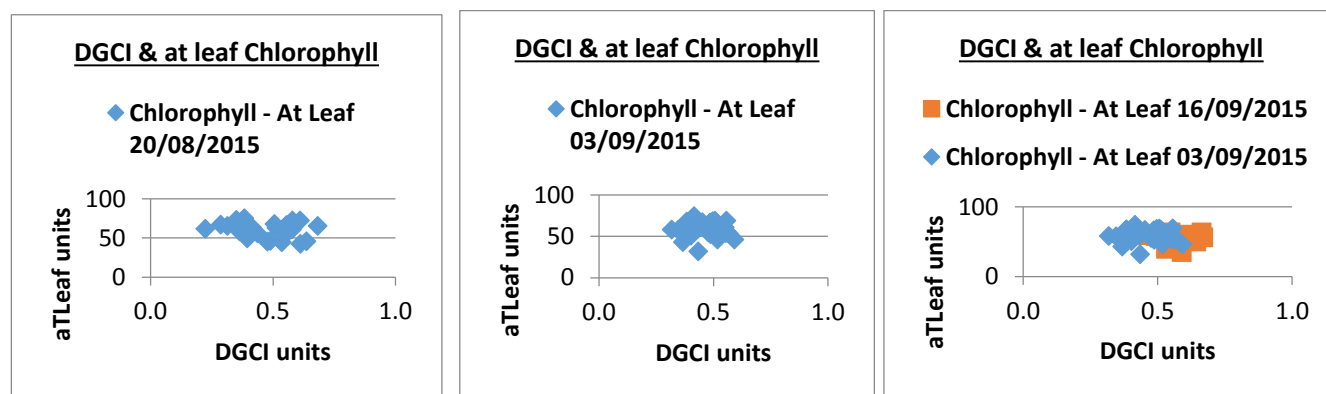


Figures 68 – 72 show comparisons for Viburnum

Skimmia

DGCI & AT Leaf Chlorophyll (Figure 73 & 75)

As found in the plant species Viburnum, the Green index app DGCI measure of dark green colour index and the chlorophyll measure from the AT Leaf chlorophyll meter was found to correlate also for the plant Skimmia during both the mid season growth period.

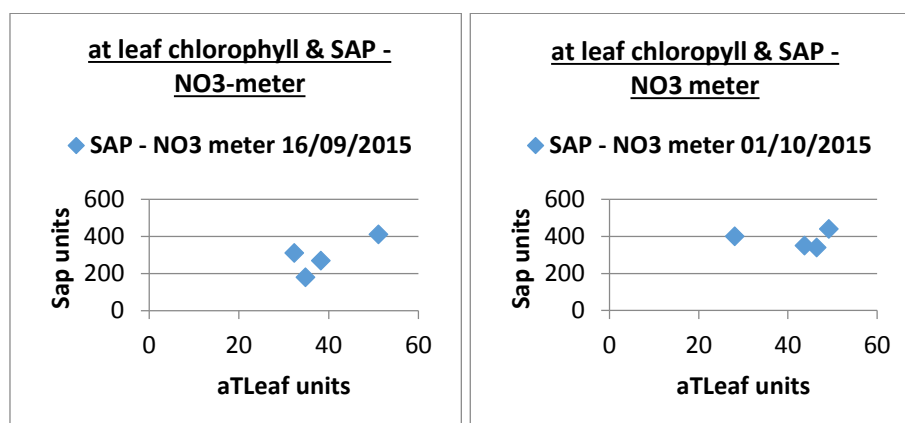


Figures 73 – 75 show comparisons for Skimmia

Chamaecyparis

AT Leaf Chlorophyll & SAP No3 meter (Figure 76 & 77)

Correlation was found between the AT leaf chlorophyll meter and the SAP meter during the late growth season. A correlation was found, the nitrogen level dropped (the SAP meter measure) with the chlorophyll measure following the same.



Figures 76 – 77 show comparisons for Chamaecyparis

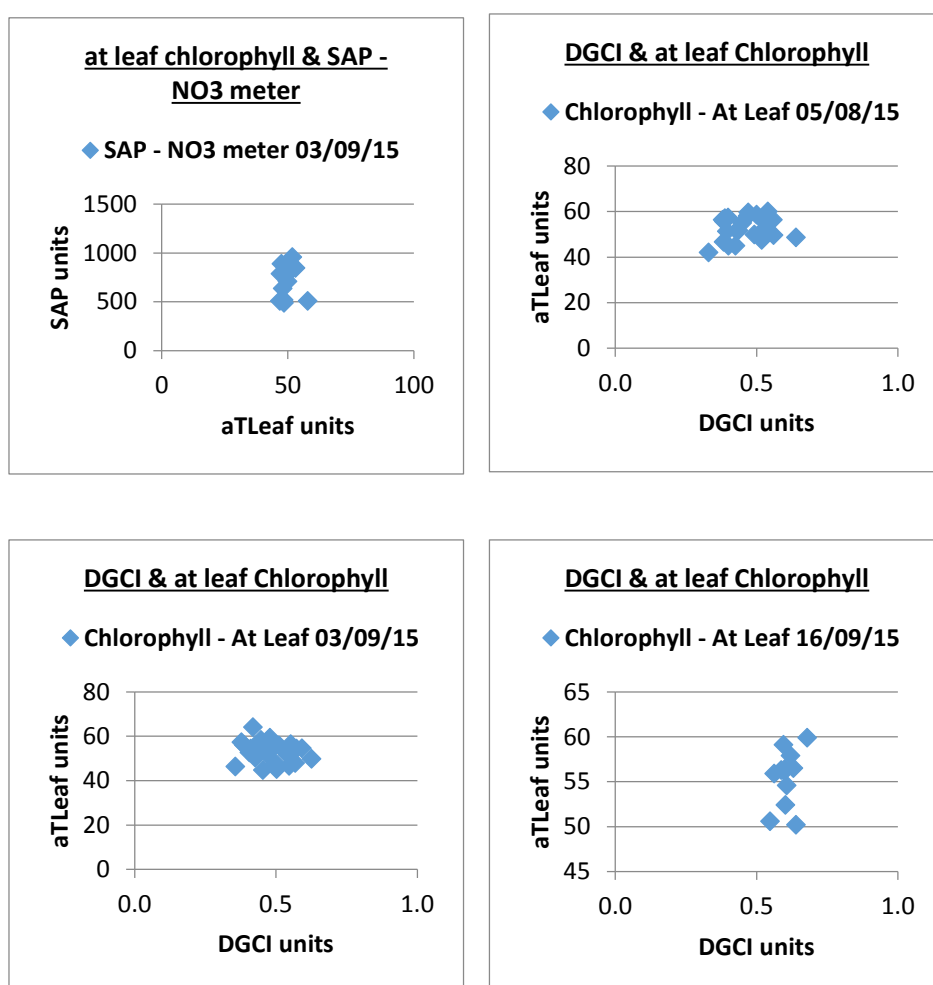
Buddleja

AT Leaf Chlorophyll & SAP No3 meter (Figure 78)

As seen above with the Chamaecyparis in late growth season a correlation was found between the AT leaf chlorophyll meter and the SAP meter during the mid-growth season. A correlation was found, the nitrogen level dropped (the SAP meter measure) with the chlorophyll measure following the same.

DGCI & AT Leaf Chlorophyll (Figures 79-81)

As found in the plant species Viburnum and Skimma the Green index app DGCI measure of dark green colour index and the chlorophyll measure from the AT Leaf chlorophyll meter was found to correlate during both the mid and late season growth period.



Figures 78 – 81 show comparisons for Buddleja

Appendix 12 – Statistical Analysis of Results

1. As expected highly significant differences were found between plant heights in different compost treatments, especially in the plant species *Buddleja*, and to a lesser extent in *Viburnum* later in the growing season. No significant difference was found in plant height for the species *Chamaecyparis* however observations found visual effects.
2. In regard to compost EC highly significant differences were found between compost treatments in mainly the *Skimma* species especially in the early growth season. None was found in the plants species *Viburnum*.
3. SAP NO₃ meter nitrogen measurement was found to differ highly significantly between compost treatments in the plant species *Skimma* in the mid- to late growth season. No significance in the other plant species and the early growth season for *Skimma*.
4. Highly significant differences were found between compost treatments in the atLEAF⁺ Chlorophyll meter results in the plant species *Skimma* in the mid growth season and in the late growth season for the *Buddleja* plant species.
5. Compost conductivity and compost total nitrogen was found to be highly significantly different between compost treatments in the three species *Viburnum*, *Skimma* and *Chamaecyparis*. Not as significant in the plants species *Buddleja*.
6. Tissue % nitrogen was found to be highly significantly different between compost treatments in species *Chamaecyparis* and *Buddleja*.
7. Drain water conductivity and total nitrogen was found to have a highly significant differences between compost treatments in all four plant species in the early growth and late growth season. Less significance was found in the mid growth season.

The table below shows the level of significance across all four varieties and each replicate for both PCS and Greenmount combined. The numbers relate to the specific dates on which the readings were taken.

ns – not significant * - significant ** - highly significant *** - v. highly significant

Variate Number	Variate Name	Plant Species				Variate Number	Variate Name	Plant Species			
		Viburnum	Skimmia	Chamaecyparis	Buddleja			Viburnum	Skimmia	Chamaecyparis	Buddleja
11	heights cm 1	ns	ns	ns	ns	82	G Index DGCI 1	*	ns	ns	**
12	heights cm 2	ns	ns	ns	**	83	G Index DGCI 2	ns	*	ns	ns
13	heights cm 3	ns	*	ns	*	84	G Index DGCI 3	ns	***	ns	***
14	heights cm 4	ns	ns	ns	***	85	G Index DGCI 4	**	ns	*	ns
15	heights cm 5	*	ns	ns	***	86	G Index DGCI 5	**	ns	**	ns
16	heights cm 6	**	ns	ns	**	87	G Index DGCI 6	***	ns	ns	*
17	heights cm 7	**	ns	ns	***	94	Compost COND us/cm 1	***	**	**	**
18	heights cm 8	ns	*	ns	***	95	Compost NH ₄ - N mg/l 1	*	*	ns	*
19	heights cm 9	*	*	**	***	96	Compost NO ₃ - N mg/l 1	***	***	**	**
51	EC ds/m 1	ns	**	**	ns	97	Compost TON mg/l 1	***	**	*	**
52	EC ds/m 2	ns	***	*	*	101	Compost COND us/cm 2	**	***	***	*
53	EC ds/m 3	ns	**	ns	ns	102	Compost NH ₄ - N mg/l 2	ns	ns	ns	ns
54	EC ds/m 4	ns	**	ns	**	103	Compost NO ₃ - N mg/l 2	**	***	**	**
55	EC ds/m 5	ns	ns	ns	ns	104	Compost TON mg/l 2	**	***	**	*
56	EC ds/m 6	ns	*	ns	ns	108	Compost COND us/cm 3	**	**	**	ns
57	average EC ds/m	*	**	*	*	109	Compost NH ₄ - N mg/l 3	ns	ns	ns	ns
66	SAP NO ₃ meter 1	**	ns	ns	ns	110	Compost NO ₃ - N mg/l 3	***	**	***	ns
67	SAP NO ₃ meter 2	ns	ns	*	ns	111	Compost TON mg/l 3	***	**	**	ns
68	SAP NO ₃ meter 3	*	ns	ns	ns	115	Compost COND us/cm 4	***	***	*	***
69	SAP NO ₃ meter 4	ns	***	ns	ns	116	Compost NH ₄ - N mg/l 4	*	ns	**	**
70	SAP NO ₃ meter 5	ns	***	ns	ns	117	Compost NO ₃ - N mg/l 4	**	***	ns	***
71	SAP NO ₃ meter 6	ns	ns	ns	**	118	Compost TON mg/l 4	***	***	*	***
72	SAP NO ₃ meter 7	ns	***	**	ns	135	Water COND us/cm 1	*	***	**	***
73	SAP NO ₃ meter 8	ns	ns	*	ns	136	Water NO ₃ -N mg/l 1	**	***	**	***
74	Chlorophyll At leaf 1	ns	**	ns	ns	140	Water COND us/cm 2	**	*	*	ns
75	Chlorophyll At leaf 2	ns	ns	ns	ns	141	Water NO ₃ -N mg/l 2	**	*	*	ns
76	Chlorophyll At leaf 3	ns	**	ns	**	145	Water COND us/cm 3	**	ns	*	**
77	Chlorophyll At leaf 4	ns	**	ns	ns	146	Water NO ₃ -N mg/l 3	ns	ns	*	*
78	Chlorophyll At leaf 5	ns	**	ns	***	150	Water COND us/cm 4	**	**	***	**
79	Chlorophyll At leaf 6	ns	ns	ns	***	151	Water NO ₃ -N mg/l 4	**	**	***	*
80	Chlorophyll At leaf 7	ns	ns	ns	**						
81	Chlorophyll At leaf 8	ns	ns	ns	***						