

'National Project on the Impacts of Climate Change on Ecosystem Function'

Data collection protocols for the Walpeup FACE rings

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WALPEUP AGFACE SITE

35°07'20"S, 142°00'18"E

Field Layout

Walpeup Plot Layout – 2008/2009

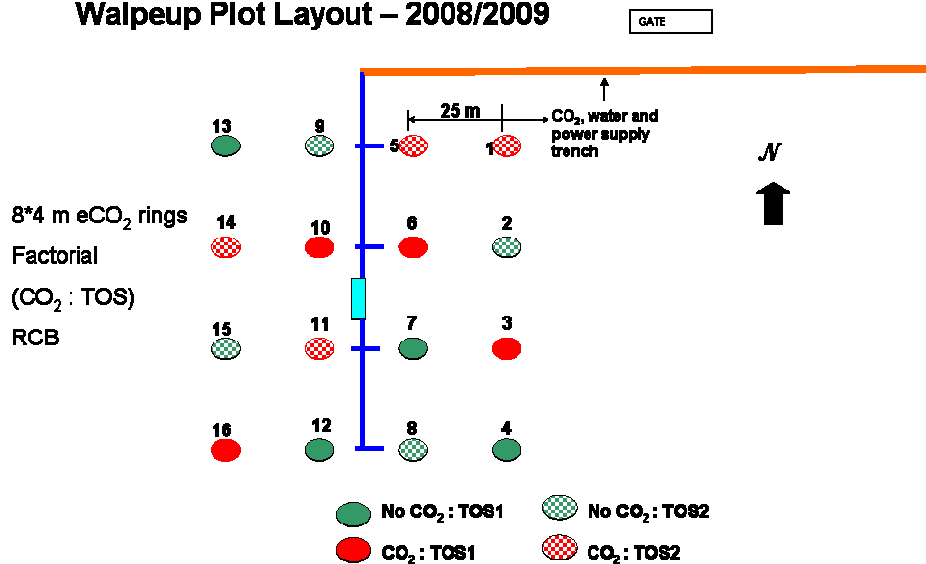


Figure 1 – field layout at the Walpeup Mallee Research Station AGFACE site, 2008.

Data collection protocols – Plant Sampling

Even though much data can be collected non-destructively (eg canopy temperatures, ground cover, water use, phenology, various vegetation indices such as NDVI), there is a need to take some destructive samples at key stages. For an annual crop, this is usually at the floral initiation, anthesis and then maturity. Because of this, destructively harvested subplots will be sown for each treatment. Table 1 provides a summary of the destructive sampling.

The experimental areas are small and this limits the amount of destructive sampling that can be done. It is proposed to take destructive samples three times during the crop and to then “back-fill” the data set with interpolated data taken from non-destructive samplings taken more frequently.

- Emergence

Emergence counts taken – 4 x 0.5 m row = 2 m of drill row once the crop has emerged. Take care to ensure that the plots are not damaged. These are to be taken in the ring experimental area.

Data: Plant number per m of drill row

- **Early Plant N sample**

At the 3-5 leaf stage, take 10 plants at random from each ring. The actual growth stage to be recorded and the plant roots cut off and the shoot separated into the rhizome (white part below ground) and the above ground portion of leaves called the pseudostem. Measure the leaf area of the pseudostem and then dry these samples separately and record dry weight, grind and store for N analysis.

Data: Rhizome dry weight (10 plants), Pseudostem dry weight (10 plants), Leaf area (10 plants).

- **FACE Biomass Sampling Protocol DC30**

In the 'DC30 growth' section of each ring, 4 x 50 cm rows will be sampled. Rows will be together and be representative of the plot. Take samples from the north west quadrant of the ring (see diagram).

HARVESTING PROCEDURE

1. Remove plants by the roots along each 50 cm row – i.e. pull up carefully. Remove dirt adhering to roots/subsurface tissue – DO NOT WASH.
2. Immediately place into a labeled paper bag in an esky with ice packs. Keep samples as cool and dark as possible. Do not expose to full sun once sampled.
3. Once back at the lab place samples into a refrigerator (4°C).
4. Count the number of plants in each sample and record on the data sheet.
5. Weigh and record the fresh weights of the plant material in the whole sample.

Data: Total fresh weight, subsample fresh weight, total sample plant number,

SUBSAMPLE

1. Subsample plants taking at least 30% of the bulk sample size, and record fresh weight.
2. Count the number of plants in the subsample.
3. Count the number of shoots (main stems plus tillers) in the subsample.
4. Separate the plants into pseudostem (above ground green part), rhizome (underground stem white part) and roots. Cut the shoot where it turns green – green part = pseudostem, white part = "rhizome". Remove the leaves from the pseudostem by cutting at the ligule.
5. Measure leaf area of leaves, measure area of pseudostem.
6. Keep root, leaf, rhizome and pseudostem samples separate.

7. Dry samples at 70°C for 3 days and record dry weights.
8. Grind leaf, rhizome and pseudostem samples for N, WSC and fibre analysis.
9. Ground subsamples to be placed in labeled glass vials for storage until analyses are undertaken.

Data: subsample plant number, subsample shoot number, subsample leaf area, subsample leaf dry weight, subsample rhizome dry weight, subsample pseudostem dry weight.

BULK SAMPLE (what is left after the 30% subsample is removed)

1. Remove fully expanded leaves at ligule, cut shoots where they turn green and keep the rhizomes, leaves and pseudostems separate. Roots can be discarded.
2. Dry samples at 70°C for 3 days and record dry weights.
3. Retain these samples as back up for N, WSC and fibre analysis and archive. It is not necessary to grind these samples yet.

Data: Bulk sample rhizome dry weight, Bulk sample leaf dry weight, Bulk sample pseudostem dry weight.

SPAD and Chlorophyll Procedure. To be done on intact plants in the field not on the sample collected.

1. Select a plant main shoot – youngest fully expanded leaf.
2. Take one mid leaf recording per leaf.
3. Remove leaf from plant at the ligule.
4. Repeat sampling procedure to measure and take 10 leaves per ring.
5. Bulk the 10 leaves for each sample, place in plastic zip bag and into cold esky.

Freeze all samples for later chlorophyll analysis.

Data: SPAD meter readings from 10 leaves, 10 leaves.

CANOPY INTERCEPTION

Record 3 ceptometer readings at ground level in the non-destructive sampling area of each ring. Ensure the instrument reads 100 in full sun above the canopy. Make sure the area sampled is not where any other destructive sampling was done and is away from the ends of the plots.

Data: 4 ceptometer readings per ring.

• **FACE Biomass Sampling Protocol - DC65**

In the 'DC65 growth' section of each ring, 4 x 50 cm rows will be sampled. Take samples from the south west quadrant of the ring (see figure 2).

HARVESTING PROCEEDURE

1. Pull up plants along each 50 cm row.

2. Keep samples as cool and dark as possible. Do not expose to full sun once sampled.
3. Once back at the lab place samples into cool storage (4°C) until processing can occur. Please do as promptly as possible though.
4. Remove any soil, roots and weeds.
5. Count the number of plants in each sample and record on the data sheet.
6. Weigh and record the fresh weights of the plant material in the whole sample.

Data: Number of plants in bulk sample, sample fresh weight, subsample fresh weight.

SUBSAMPLE

1. Subsample plants (approx 15-25% of the bulk sample size – minimum of 10 plants) and record fresh weight. Record the number of plants in the subsample.
2. Count the number of heads in the subsample.
3. Separate the heads, leaves and stems. Leaves to be removed at the ligule.
4. Measure leaf area of leaves, measure area of stems.
5. Keep stem, head and leaf samples separate.
6. Count the number of florets per head – do 20 heads per subsample taken at random.
7. Dry samples at 70°C for 3 days and record dry weights.
8. Grind head, leaf and stem samples for N, WSC and fibre analysis (to be done later).
9. Ground subsamples of each component (leaf, head, stem) to be kept separate and placed in labelled glass vials for storage until analyses are undertaken.

Data: Number of plants in subsample, Number of heads in subsample, leaf area of subsample, number of florets in 20 heads, subsample stem dry weight, subsample leaf dry weight, subsample head dry weight.
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BULK SAMPLE

4. Bulk material can be discarded.

SPAD and Chlorophyll Procedure. To be done on fresh plants in the field

6. Select a plant main shoot – youngest fully expanded leaf.
 7. Take one mid leaf recording per leaf.
 8. Remove leaf from plant at the ligule.
 9. Repeat sampling procedure to measure and take 10 leaves per plot.
 10. Bulk the 10 leaves for each sample, place in plastic zip bag and into cold esky.
- Freeze all samples for later chlorophyll analysis.

Data: SPAD meter readings from 10 leaves, 10 leaves.
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CANOPY INTERCEPTION

Record 4 ceptometer readings at ground level in the non-destructive section of each ring. Ensure the instrument reads 100 in full sun above the canopy. Make sure the area sampled is not where any other destructive sampling was done and is away from the ends of the plots.

Data: 4 ceptometer readings per ring.

ROOTING DEPTH

(need soil corer – either Peter (UM sampler) or Russel (DPI sampler)) or Walpeup DPI – record sampler and also sampling tube diameter.

In the DC65 sampled area within the rings, we would like to make an assessment of rooting depth and also root biomass. Please take care with locating the sampler and moving around the plots. A core to at least 1.5 m (use discretion and local knowledge) is to be taken and cut, washed and assessed. The samples can be kept in a cool room for 6-8 weeks prior to washing and scanning but is best done as soon as possible.

1. Take 2 cores per plot within the DC65 sampled area of the ring.
2. Cores to be taken to 1.5 m deep one core in the row and the other between the rows – these two to be composited.
3. Cores to be cut into 0-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, >140 mm – same depths as the neutron probe readings and put into sealable plastic bags (freezer bags).
4. Cores can be stored in the cool room until root extraction.
5. Cores then to be washed to extract roots – difficult job in the clay but can be done. The means the cores should not be stored for more than a few weeks before washing to extract the fresh roots.
6. Once washed and extracted, stored roots in 10% ethanol in preparation for scanning.
6. Roots to be scanned after root washing to assess root lengths.
7. Once scanned, roots to be dried and weighed.

Data: Root dry weight per sample depth, root length per sample depth.

• **FACE Biomass Sampling Protocol – DC90**

In the 'DC90 maturity' section of the ring 6 x 100 cm rows will be sampled per ring as a block. This is to be in the north east quadrant of the ring.

HARVESTING PROCEEDURE

1. Grain harvest to be done at physiological maturity – when the grain is hard and the plants have senesced. This will differ between sowing times.
2. Pull up plants within the 6 x 100 cm row.
3. Bulk the sample and immediately place into a large white poly bag.
4. Samples can be stored in a glasshouse or similar weather proof dry area until processing. These samples should not need additional drying.

5. Trim the roots back to the plant crown and remove any soil adhering.
5. Count the number of plants in the bulk sample and weigh the bulk sample.

Data: Plant number per bulk sample, plant fresh weight per bulk sample.

SUBSAMPLE – at the lab

1. Take 20 plants from the bulk sample for dissection.
2. Weight and record the weights of the 20 subsampled plants.
3. Count the number of heads in the subsample.
4. Separate the heads and stems. There should be no green leaf material left.
5. Keep stem and head samples separate and weigh the heads.
6. Hand thresh the heads from the 20 plants and weigh and record the seed sample weight. The difference between the head weight and the seed weight is the chaff.
7. Count 200 seed weights from the seed sample and record.
8. Dry samples at 70°C for 3 days and record dry weights.
9. Ground sub-samples of each component (leaf, head, stem) to be divided to be placed in labelled glass vials for storage until analyses are undertaken.

Data: Weight of 20 plants, heads per 20 plants, Head weight of 20 plants, stem weight of 20 plants, Chaff weight of 20 plants, seed weight of 20 plants, leaf weight of 20 plants.
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BULK SAMPLE

Bulk material is to be threshed to get a large seed sample for further grain quality analysis.

DRY END NEUTRON PROBE CALIBRATION

1. Take a final neutron probe recording as close to soil sampling as possible.
2. As near to the access tube as possible, take a soil sample to the probe reading depths and subdivide the sample in the same way the starting sample was divided.
3. Weigh wet and before drying check and record maximum rooting depth for each core – use a core-break method.
4. Dry soils at normal temperature.

A spring sample also be taken (outside rings), providing it is wet, for calibration as sowing samples have dry subsoils

FINAL SOIL TEST VALUE

1. Take a soil core to 60 cm, subdivide into 0-10, 10-20, 20-40, 40-60. One core per half ring.
2. Sampled to be tested for soil C, N and other nutrients – at some time later – air dry and store as archived sample.

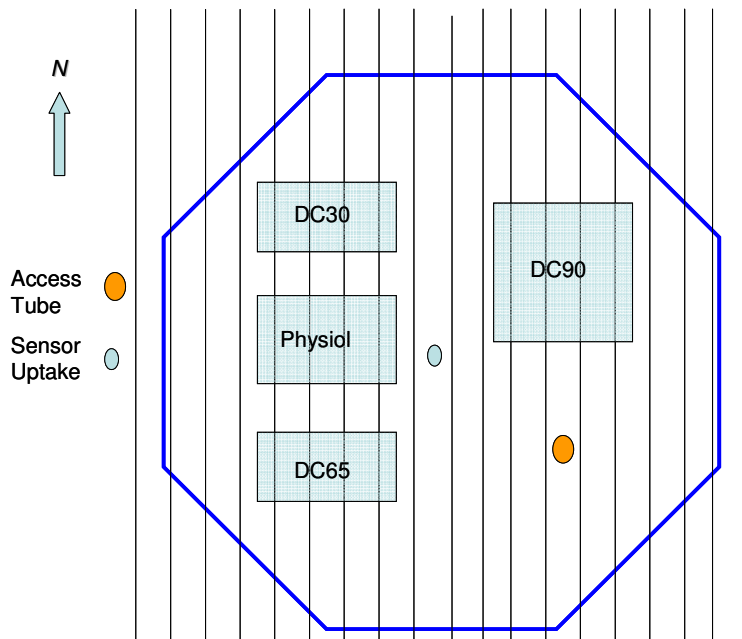


Figure 2 – Layout and location of sampling areas for AGFACE Walpeup site.

Management Issues:

N management – based on the tissue levels measured and the general health of the crop, additional N (as urea topdressed) can be added to the whole ring. Record amount added and crop stage.

Herbicide Management – avoid use of pre-emergence herbicides and for preference use post-emergence herbicides and hand weeding for weed management.

Fungicide Management – At about 8 weeks after sowing, use a protectant spray against stripe rust – the actual product to use to be advised by Grant Holloway and the pathology team at Horsham.

Insecticide Management – At about 8 weeks after sowing, use a routine spray of Confidor to prevent aphid flights infecting the crop with Barley Yellow Dwarf Virus.

Water Management – Should the season become dry, the experiment is to be watered to keep the water supply at or near a decile 5 year. If the season is very wet, then we have a problem.

Table 1. In crop measurements (predominantly destructive) that will be acquired at FACE, wheat in Horsham in 2008.

Measurement	Purpose	Frequency	Instruments	Comments
Soil Testing	Determine soil chemical status	Prior to sowing and post final harvest		Soil cores 0-10 cm & 10-20 cm for basic chemical analysis (including soil C) & 0-50cm for mineral N. Mineral N will also be determined from neutron tube cores. Soils will be archived for future reference.
Plant Establishment	Determine plant density	4-6 weeks after sowing		
Phenology	Detect & record crop growth stages to provide data and indicate timing for other measurements	On-going particularly around key growth stages (DC30, DC65, maturity)	Dissecting microscope for DC30	Border plots will be used for destructive sampling to determine DC30
Above Ground Biomass	Biomass accumulation & partitioning, plant and tiller no., leaf area & leaf N determination	DC30, DC65 and maturity	Li-3100 Area Meter or similar	Grain quality, yield and yield components will be determined on final harvest samples. All samples will be archived for future reference.
Light Interception	Provide data on radiation capture and conversion	Fortnightly and to co-incide with above ground biomass measurements	Ceptometer	Readings will be taken within 2 hours of solar noon
Rooting depth and biomass	To assess root:shoot ratios	At DC65	Hydraulic soil corer	V1N1 treatments taken from areas adjacent to Plot A or L
Soil Moisture	Growth in relation to soil moisture	NMM (20-180cm) every 4 weeks in winter & more frequently as soil moisture content declines. TDR (0-20 cm) spot readings or logged data. Readings to co-incide with above ground biomass measurements	TDR and neutron moisture meter (CPN503)	Probe calibrations will be taken pre-sowing and at harvest. Extracted soil cores will be used to determine bulk density. Soils sampled at wet (sowing) and dry (harvest) for neutron probe calibration.

Data collection protocols – Soil sampling and neutron probe readings

Presowing:

a) To assess soil water content, at the time of installation of the neutron probe access tubes, samples (to 2.0 m in 20 cm increments) to be taken and dried and these data used to calculate volumetric soil water content. One set of cores from each replicate to be taken for textural analysis using sedimentation.

b) To assess soil fertility, a standard soil test to be taken with cores to 10 cm taken with a tread sampler across the experimental areas. These samples to be composited on a replicate basis and analysed for Total Soil N, Colwell P, Skene K, Soil C, trace elements.

c) To assess available soil N, a 50 cm core per ring to be taken and these samples air dried and sent for mineral N analyses (nitrate and ammonium)

At Anthesis:

See rooting depth above under DC65 sampling.

At Maturity

Cores for the “Dry end” neutron probe calibration data taken in areas immediately adjacent to the access tube, and divided into the sample depths at 20 cm increments to 2.0 m.

Neutron Probe readings

Water use will be monitored non-destructively using the in-situ technique of a neutron probe for the profile from 20 cm to 180 cm deep. A proper calibration is essential when using a neutron probe (CPN503) and this can be achieved using a two point calibration against volumetric soil moisture contents taken pre-sowing and after harvest (see above). Access tubes will be inserted immediately after sowing using a volumetric core sampler which extracts a core of the same size as the access tube. The soil sample extracted is assessed for bulk density and soil moisture content (as well as for mineral N) down the profile and neutron probe counts taken as soon as the tube is installed to give the “wet-end” point.

Neutron probe readings should be done at each phenophase harvest (DC30, DC65 and DC90) and at mid-tillering, during stem elongation and at two equally spaced times between anthesis and maturity (about 3 weeks apart).

TDR readings

Because the neutron probe is not suitable for use in the top 20 cm layer of the soil profile, Time Domain Reflectometry (TDR) can be used to assess this parameter. This sensor is inserted and a reading taken using in real time. Because of time and distance, please take top 20 cm samples for thermogravimetric moisture contents rather than use the TDR. Take these samples from around the edges of the plot.

Data collection protocols – Non-destructive measurements

There will be several types of remotely sensed data collected at the Horsham FACE experiment in 2007. They will allow measurement of canopy temperature for water relations using an infra-red thermometer (IRT), crop cover and biomass to track growth and to interpolate between the destructive samples (Crop Circle and digital photographs), aerial and ground deployed multispectral imagers to understand spatial variations and nitrogen and water relations, light use (ceptometer), and leaf chlorophyll (SPAD). The measurements to be taken are summarised in Table 2.

Crop Circle

The Crop Circle is an “active” sensor that should be able to operate under a wide range of conditions, including cloudy conditions without affecting the readings. It will allow interpolation between destructive sampling measurements. Measurement frequency should follow crop phenology (see Table 6). Most of the other measurements will follow this schedule.

Infra-Red Thermometers (IRT)

The IRT will collect canopy temperature measurements. There will be two sampling regimes. One will measure canopy temperatures at the time of neutron probe readings. The second will record plot temperatures on a regular basis but will be adjusted depending on growth stage (see below). For each plot there will be two types of measurements, nadir and oblique. The nadir view (sensor pointed straight down looking at soil + plant) will simulate an aerial view while oblique views (standing back pointing sensor at an angle of about 25 deg towards crop) will allow plant-only temperatures to be recorded once plants reach sufficient size (~DC24). The oblique view will be acquired walking along the east side of plots pointing the IRT towards the west. Temperatures of dry and wet soil patches will also be recorded along with meteorological data. The IRT measurements will be performed weekly once the crop reaches the stage of requiring irrigation (to be determined). These data will permit development of the Water Deficit Index. Crop temperatures can only be collected under clear skies so it will be necessary to accommodate other activities so that IRT readings can be acquired once per week during critical times or during crop dry-down periods. The exact details need to be worked out as the season progresses. In all cases, IRT readings will be acquired during overflights, regardless of sky conditions.

The IRT ‘Calibration Source’ records ambient temperature and will be measured at the beginning middle and end of each run, at least. It must be kept out of direct view of the sun so the temperature on the display accurately represents the temperature of the concentric-rings black surface. These data will allow a check of the IRT and be used to correct data if needed.

Table 2. Remote sensing measurements that will be acquired at FACE, wheat in Horsham in 2008.

Instrument	Purpose	Frequency	Comments
Crop Circle	Crop cover and biomass	Once over bare soil, weekly to fortnightly depending on crop stage	Calculate Red, NIR, NDVI, SAVI
Infrared Thermometer (IRT)	Water stress	During dry-down periods, at time of neutron probe readings and over-flights, and once a week on sunny days	Oblique and nadir data
Infrared Calibration Source	Within-run calibration check	As part of IRT measurement routine, records ambient temperature	Must be kept shaded, away from direct sun in field
Imagers	Spatial variation	4 times during season: DC24 (mid-tiller), DC30 (stem elongation), DC60-65(anthesis), DC90 (harvest)	6-narrow bands and thermal imager for N, cover, biomass, water stress
Digital photographs	Cover reference,	Fortnightly/weekly	
Ceptometer	Light interception	Fortnightly/monthly?, at destructive sampling	Take within two hours of solar noon
SPAD	Surrogate for leaf chlorophyll	Fortnightly?, at destructive samplings	Determine which leaf, number leaves/plot

Table 6 Sampling times and frequencies, Horsham FACE site, 2008.

Sampling times	Target	DC growth stage	Frequency	Number of samples (approx.)
Just after sowing	Bare soil		Once	1
Late June to about mid-August	Early crop development	DC11-15	Fortnightly	3-4
Mid-August to mid October	Rapid cover increase	DC15-60	Weekly	8-9
Late Oct-Early Dec	Senescence	DC60-92	Fortnightly	3-4

Imagers

Two 3-band imagers plus one infrared imager will be part of the camera package that will acquire 7 bands simultaneously at four times during the season to understand spatial variability across the rings and build algorithms for cover, biomass, canopy nitrogen and water status. The four dates will correspond as closely as possible (weather permitting) to the three destructive sample dates plus one earlier in the season (DC24) to test for early-season N detection. Pixel resolution for the multispectral (MS) imagers will be 0.2 to 0.3m at 1000 to 1200

m above ground level (AGL). Ideally, pixel resolution should be 1/8 the size of the target. If sub-plot width is 1.6 – 1.7 m then 0.2 m pixels size is appropriate. The thermal imager resolution is about ¼ that of the MS so resolution would be near 1 m at that altitude. In order to distinguish the small plots, altitude would need to be much lower but this may not be practical, especially since at lower altitudes the sensor field of view will not capture the entire experimental site. Since N treatments are not expected to have any significant effect directly on crop water relations (except as N allows greater canopy development and therefore influences cover), resolution at the scale of the half-rings will probably be sufficient. Thus, altitude will need to be adjusted to allow pixel resolutions of about 0.7 m (5.5 m half-ring radius subtracting centre walkway).

Digital Photographs

Digital photographs are valuable records of crop conditions that can be referred to after the season to identify crop conditions or help interpret data anomalies. They will also be used as reference for ground cover to develop relationships to other spectral measures, such as the Crop Circle or Ceptometer. These will be collected at the same times as the crop circle. The number of images could become very large so during the weekly Crop Circle collection, it might be decided to collect photos fortnightly or photograph a sub-set of plots. This will depend in part on labour availability.

Ceptometer

This will be deployed as near the time of the Crop Circle measurements to develop relationships between light interception and remote measures of cover.

Calibrations

All instruments need calibration targets. The aerial multispectral images will require ground panels (Figure 4). These panels and wet and dry soil areas will also be used as reference for the ground-based sensors. Panels and support stands used in the ORL project will be provided. The thermal imagery will require dry and wet soil areas, so 8 m x 8 m soil areas will be placed to the side of one of the bays so that they are visible within the imagery and are easily accessible for ground measurements. This will allow low and high temperature measurements to be determined for use in water index development.

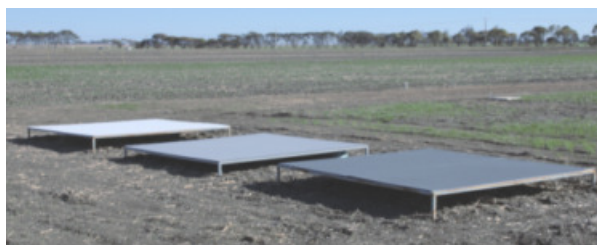


Figure 4. Painted ground reference targets (2.8 m X 2.8 m) visible in aerial imagery. Used to correct imagery and ground sensor data to reflectance.

Other comments

All measurements must be time-stamped. A digital watch can be time-synced to universal time (on the internet) before going to the field. All other instruments that record time must be synced to that time. There is no reason this shouldn't be less accurate than 1 s, but within 5 s is acceptable. All measurement locations must be known. This can consist of noting the ring number and plot letter or, if necessary, the X and Y distance from a corner or centre post, depending on the accuracy required. This latter is recommended for destructive sampling. This will allow precise location in the imagery.