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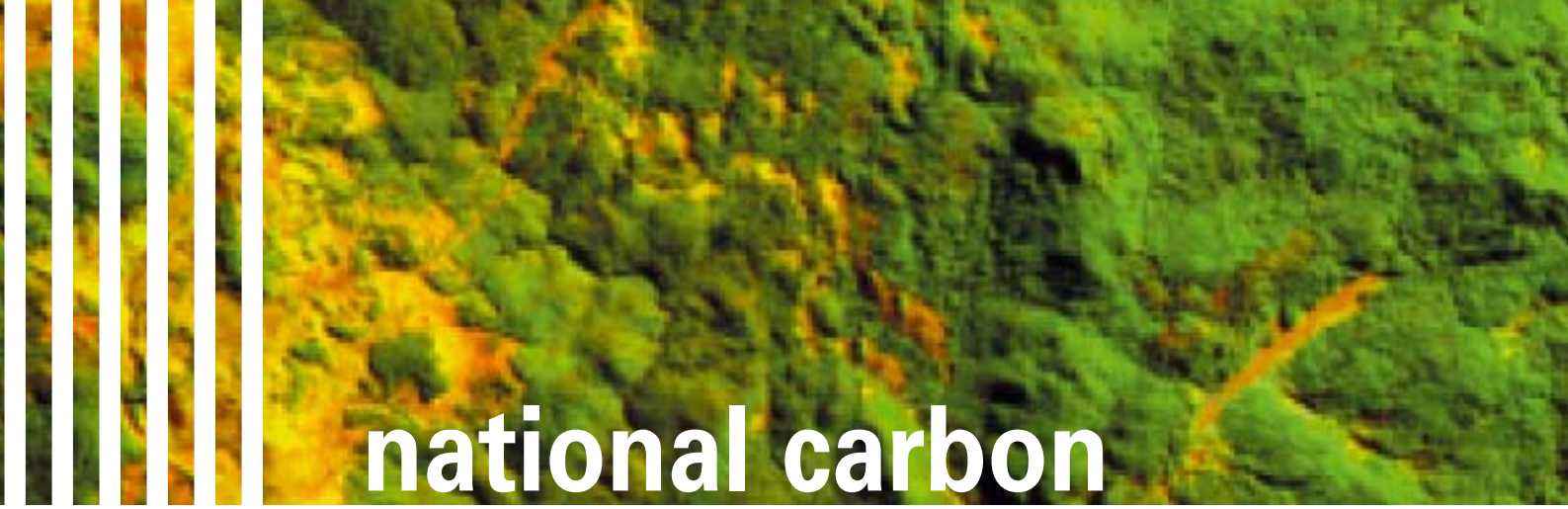

national carbon accounting system

technical report no. 31

Protocol for Sampling Tree and Stand Biomass

Peter Snowdon, John Raison, Heather Keith, Peter Ritson, Pauline Grierson, Mark Adams, Kelvin Montagu, Hui-quan Bi, William Burrows and Derek Eamus

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PROTOCOL FOR SAMPLING TREE AND STAND BIOMASS

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National Carbon Accounting System
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March 2002

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March 2002

Environment Australia Cataloguing-in-Publication

Protocol for sampling tree and stand biomass / P. Snowdon … [*et al.*]

p. cm.

(National Carbon Accounting System technical report; no. 31)

ISSN: 14426838

1. Trees-Carbon content-Measurement. 2. Roots (Botany)-Carbon content-Measurement. 3. Forest biomass-Measurement. I. Snowdon, P. II. Australian Greenhouse Office. III. Series

333.9539'015308-dc21
333.9539'099435-dc21

SUMMARY

Estimation of the biomass and carbon content of Australian forest and woodland ecosystems and its rate of change is an important goal of the National Carbon Accounting System. This protocol describes field methods for estimating above-ground and root biomass of woody vegetation. It complements the protocols developed for estimating carbon in soils and litter (McKenzie *et al*. 2001), and the synthesis of national information on wood density (Ilic *et al*. 2000).

Ecosystem carbon is usually partitioned into four major pools:

- Carbon in above-ground biomass of live trees;
- Carbon in woody and other debris;
- Carbon in root systems; and
- Soil carbon.

It is important that boundaries between the different pools are clearly defined so that when estimating carbon stocks in an ecosystem all sources of carbon are counted and they are counted only once. Clear definitions are particularly important when the various components are estimated in separate studies, and when this protocol for sampling above-ground and root biomass is used in conjunction with the protocol developed for estimating soil carbon (McKenzie *et al*. 2001).

Two basic methods for estimating above-ground biomass of individual trees are described. In the complete harvest method the total weight of all components of the sample tree is weighed in the field and then dry weight is estimated by adjusting for the moisture content determined in sub-samples. Partial harvest methods are also outlined in which estimation of biomass is based on either sub-sampling methods or on the development and application of regression estimators that relate biomass to simple measures such as diameter, or other estimators to simple (eg. diameter) measures of component size. Generalized modifications of these methods are described for estimation of biomass of understorey shrubs and individual trees with irregular form. Two case studies are provided as examples.

Methods are described for the excavation and measurement of biomass of root crowns and major roots of individual trees. Complementary methods are given for sampling small and/or distal roots. Four case studies are provided as examples.

Statistical considerations when choosing sample trees are discussed. Details are given of various regression and ratio estimators that can be used to calibrate relationships between simple measures and component biomass. Methods for the development of expansion factors suitable for estimating stand-level biomass from inventory data are also given.

Methods are also outlined for measuring carbon concentration in the various components of biomass.

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DEFINING AND MEASURING FOREST CARBON POOLS

1.1 PREAMBLE

One of the objectives of the National Carbon Accounting System (NCAS) is to estimate the carbon stock and change in stock of Australian forests and woody ecosystems. This requires estimates of above-and below-ground carbon content in these communities. Protocols for sampling and estimation of soil and litter carbon (McKenzie *et al*. 2001) and wood density (Ilic *et al*. 2000) have been developed for the NCAS. The protocol developed here primarily addresses methods for sampling and measurement of above- and below-ground (root) components in woody vegetation.

Inventory of forest carbon depends directly or indirectly on the estimation of biomass in sample plots. Different sampling strategies are likely to be required depending on the structure, composition and scale of the various stands involved; the specific objectives of the inventory; and the financial and other resources available for its completion. The underlying objective should be to choose the sampling method or combination of sampling methods that will result in the most efficient collection of reliable information at the requisite scale. Further information on inventory and field measurement procedures for carbon accounting are given by GRO (2001).

Destructive sampling of all vegetation within an individual plot may be feasible for grass and shrub vegetation but is rarely used for forest ecosystems. Biomass of large vegetative structures is usually estimated by applying ratio or regression methods to an easily measured variable, such as stem diameter, which has a strong relationship with biomass of the component of interest. The ratio or regression relationships may be known from a previous study or may need to be developed. Details for the development and application of ratio and regression methods are provided in this protocol.

1.2 CARBON POOLS WITHIN ECOSYSTEMS

Ecosystem carbon is usually partitioned into four major pools:

- Carbon in above-ground living biomass;
- Carbon in woody and other debris;
- Carbon in root systems; and
- Soil carbon.

A summary of these and their major sub-compartments is given in Table 1. As indicated, methods for estimating some components have been given in the protocol developed for sampling soil carbon (McKenzie *et al*. 2001).

Table 1. Compartments used for inventory of carbon in forest ecosystems indicating those components covered (✓) or partly covered (P✓) by the protocol for estimating soil carbon content.

Compartment	Soil protocol	Sub-compartment
Above-ground biomass		Overstorey biomass
		Understorey biomass
Woody debris		Standing and suspended woody debris
	✓	Fallen woody debris
Roots systems	P✓	Fine roots (< 2 mm only)
	P✓	Live woody components
	P✓	Dead woody components
Soil	✓	Fine surface litter
	✓	O horizons
	✓	< 2 mm fraction of mineral soil
	✓	> 2 mm mineral fraction
	✓	> 2 mm organic fraction (excluding root material if counted elsewhere)
Other		Fine suspended debris
		Buried non-root woody material > 50 mm diameter

It is important that the boundaries between the different components are clearly defined so that when an estimate of carbon stocks of an ecosystem is made all sources of carbon are counted and that no source is counted more than once. Clear definitions are particularly important when the various components are estimated in separate studies.

Boundary conditions which require careful definition are those separating live and dead woody roots (including stumps) between the soil and root compartments; and those defining the partition of above ground woody debris, including stumps and snags, between the soil and above-ground compartments.

1.2.1 ABOVE-GROUND LIVE BIOMASS AND CARBON

The above-ground pool of ecosystem carbon is comprised of:

- Biomass of the above-ground components of overstorey species; and
- Biomass of the above-ground components of understorey species including grasses, forbs, shrubs and juveniles of the overstorey species.

Dead leaves, branches and other material physically attached to the living component, eg. epiphytes, are counted as part of the above ground live biomass.

1.2.2 ABOVE-GROUND WOODY DEBRIS

Above-ground woody debris is composed of woody material including:

- Individual pieces (twigs, branches, logs) lying in contact with the surface soil, litter or other component of debris;
- Heaps and windrows;
- Snags (standing dead components) and stumps (lower boles retained after harvesting) with roots persisting in the soil; and
- Partially or fully suspended material.

Methods for estimating the first two components of above-ground woody debris are included in the protocol for estimating soil carbon (McKenzie *et al*. 2001). Both the above-ground and below-ground

components of stumps, upright snags and partially suspended snags are amenable for estimation by regression on diameter and/or height measurements and these two components should be separated. It may also be useful to account for upright and suspended debris separately from fallen and heaped material because it is likely that they will have different decay rates. Estimation of fully suspended detached debris will be difficult but will usually be inconsequential.

1.2.3 ROOTS

The root compartment of ecosystem carbon is comprised of:

- Fine (here defined as < 2 mm) roots;
- Woody roots and root-boles; and
- Other specialized, below-ground (or below-water) structures such as lignotubers or pneumatophores not accounted elsewhere.

Only fine (< 2 mm) roots are well sampled using the routine soil sampling protocol (McKenzie *et al*. 2001). Larger roots are poorly sampled because:

- Root distribution is related to distance from stems or stumps;
- Soil sampling is difficult adjacent to tree boles and large stumps; and
- Soil corers have difficulties cutting through live or poorly decomposed roots > 25 mm diameter.

McKenzie *et al*. (2001) defined fine roots as < 2 mm diameter, and a similar definition is used in this protocol.

It is feasible to estimate the below-ground biomass and carbon content of live roots from root-to-shoot ratios or from regression relationships with above-ground bole diameters. Regression relationships on bole diameter could also be used to estimate dead root biomass when they are still attached to a stump or snag. In fire-free environments, a good correlation may also exist between above-ground woody debris and dead coarse roots, as their relationship is largely controlled by the structure of living trees (Harmon and Sexton 1996). There is probably no practical way to estimate very large woody roots that have been detached from their stump or snag by decomposition, fire or mechanical disturbance.

Problems also lie in the distinction of live and dead roots, especially dead roots generated during land clearing operations, and in their allocation to the appropriate ecosystem compartment. It is probably impractical to separate live and dead fine roots for inventory purposes. Live woody roots tend to increase in mass as they grow with time while dead woody roots tend to decrease in mass as they decompose. Thus, they need to be treated in different ecosystem compartments. It is sometimes suggested that dead roots are treated either as coarse woody debris or, alternatively, dealt with in conjunction with soil carbon calculations (IPCC 1997; Section 5.4.2). It is probably best to assign dead woody roots, root-boles and specialized under-ground organs to their own compartment unless they have been physically removed from beneath the soil surface, eg. by root raking. If roots have been so removed they clearly become part of the above-ground coarse woody debris.

1.2.4 SOIL ORGANIC CARBON

The protocol for measurement of soil organic carbon (McKenzie *et al*. 2001) includes carbon in all dead organic matter at the soil surface for sites where it occurs. This includes carbon present in:

- Coarse woody debris (logs, branches, stumps and large pieces of charcoal);
- Surface litter (leaves, twigs, branches, bark, seeds, insect detritus, animal manure and other comminuted organic matter); and
- Horizons as defined by McDonald *et al*. (1990).

Below-ground the protocol includes carbon present in:

- The fine earth fraction (< 2 mm) of the soil;
- The coarse (> 2 mm) mineral fraction, including charcoal and gravel;
- Coarse organic debris comprising dead roots, twigs, fungal mats and other material; and
- Live roots < 2 mm in diameter.

Existing guidelines for carbon accounting (IPCC 1997) refer only to the upper 0.30 m of the soil profile. Nevertheless it is desirable to attempt to characterise the soil to a depth where a root-impeding layer is encountered (McKenzie *et al*. 2001).

1.3 METHODS FOR ESTIMATING FOREST BIOMASS AND CARBON

1.3.1 OVERSTOREY SPECIES

Biomass, and subsequently carbon storage, of individual trees or stands are estimated by applying pre-determined statistical relationships to simple tree measurements such as stem diameter and tree height. Individual tree measurements should be made according to standard forestry protocols (eg. Ellis and Hayes 1997, Wood *et al*. 1999). All stems greater than the minimum specified diameter should be measured. Smaller stems should be assigned to the understorey component of the ecosystem. Individual trees should be measured for all those parameters used as input to the predicting equations. These usually include diameter at breast height but other measurements such as tree height, stem diameter at the base of the green crown, crown diameter, bark thickness, bole length, crown length, etc. may also be used. Notes should be made of any unusual constraints to growth, physical damage and presence of hollows (eg. Eyre *et al*. 2000) which may indicate a need to adjust estimates made by the predicting equations.

Several different methods for estimating biomass from simple measurements are in common usage:

- applying a regression equation specific to the individual tree species to diameter and, sometimes, other tree measurements;
- applying a generic regression equation to diameter and, sometimes, other tree measurements;
- estimation from species-specific or generic biomass yield tables based on diameter and, sometimes, height measurements; and
- use of standard yield tables to estimate stem volume and then application of a density factor to convert to stem biomass. An expansion factor is then applied to estimate whole tree biomass.

Examples of generic and specific regression equations and expansion factors are given by Snowdon *et al*. (2001). Estimates of biomass may need to be corrected for bias (see Appendix 3) particularly if a logarithmic version of an allometric equation has been used during the estimation procedure. *Ad hoc* correction for the presence of hollows or major deformities in individual trees may also be warranted. The rational for these should be presented together with the uncorrected estimates.

When no suitable relationship exists the methods described in Section 2 can be used to measure the biomass of individual trees which can then be used to construct a new relationship using methods outlined in Appendix 3.

1.3.2 UNDERSTOREY SPECIES

Direct weighing of samples from fixed area quadrats is often used to make estimates of biomass of woody understorey shrubs. Estimates can also be gained by applying regression relationships to simple measurements such as crown diameter or height. Few such relationships have been published for Australian species. Methods for estimating

understorey biomass by direct harvest are given in Appendix 1. These can be extended to allow the construction of regression relationships applicable to particular species or ecosystems.

1.3.3 WOODY DEBRIS AND LITTER

Methods for estimating surface litter and woody debris on the forest floor have been given by McKenzie *et al*. (2001).

Biomass in stumps and stags also needs to be estimated. Diameters and heights should be measured and degree of deterioration of stumps and stags should be recorded (eg. Eyre *et al*. 2000). Biomass of stumps can be estimated by calculating volume, applying a wood density factor and then making an adjustment for degree of deterioration. Biomass of stags can be estimated by applying equations developed for live trees (if necessary after correction of diameters for losses due to shed bark) and then applying a correction for the degree of deterioration.

1.3.4 ROOT SYSTEMS

Measurement of root biomass is time consuming and expensive even to obtain moderate levels of precision. It is sometimes possible to apply a regression equation to stem diameter or some other measure of tree size in order to estimate root biomass. Sometimes a simple root:shoot ratio is used. Details of methods for direct sampling of roots or to develop local regression equations are given in Section 3. No destructive sampling should be carried out in permanent sample plots on which growth is to be remeasured.

1.3.5 SOILS

Details for estimating soil carbon are given by McKenzie *et al*. (2001). Care must be taken to carefully define the boundaries of the soil carbon pool with that of the root and woody debris pools.

1.4 CONVERSION FROM BIOMASS TO CARBON

With the exception of soils it usual to estimate the quantity of most compartments as ovendry biomass and then apply an appropriate factor to convert these estimates to estimates of carbon content. Sometimes a simple generic multiplier (typically 0.5) is used but it is more appropriate to conduct laboratory analyses for carbon concentration in each of the major components sampled during the inventory (see Sections 2 and 3).

SECTION 2

ESTIMATION OF ABOVE-GROUND BIOMASS OF INDIVIDUAL TREES

2.1 INTRODUCTION

This section outlines methods for estimation of above-ground biomass and carbon using either complete or partial harvest of sample trees. Usually this will require destructive sampling though, in some cases, sub-sampling that does not destroy the tree will be possible.

Destructive sampling will give the most accurate estimate of tree biomass but, is not practical for broadscale inventory application. Estimates obtained from destructive sampling are used to develop equations that predict biomass from easily measured variables such as stem diameter, tree height or crown dimensions. It is important that the trees chosen for sampling are representative of the populations to which the predictive equations will be applied (See Appendix 3).

2.2 INITIAL MEASUREMENTS

All candidate predictor variables for estimating biomass of individual trees or components of trees should be measured initially. This should include the variables discussed in the remainder of this section. More detail on methods for forest measurement are given by Carron (1968), Wood *et al*. (1999) and GRO (2001).

2.2.1 STEM DIAMETER

Commonly diameter at breast height (1.3 metres), i.e. DBH, is measured (this is often referred to as DBHOB to indicate that the measurement is taken over-bark). However, measurement at a height of 10 cm (d_{10}) or 30 cm (d_{30}) is also common, especially on small trees and shrubs or trees prone to forking below 1.3 m diameter. Diameter at crown break (d_{cb}) is another often useful predictor variable, particularly for estimating crown biomass, and should also be measured.

Where there is > 1 stem on a sample tree at a measurement height the diameter equivalent (d_e) of all stems may be calculated as the diameter of a circle of area equal to the sum of cross-section areas of all stems at that height

$$d_e = \sqrt{\sum_{i=1}^{n} d_i^2} \qquad (2.1)$$

where d_i = diameter of the ith stem at the measurement height.

All diameters should be measured both over- and under-bark. To measure diameter under-bark the bark can be removed from destructively harvested stems, at least at the measurement heights, to allow measurement with standard diameter tape. Alternatively, measurements of bark thickness can be made with a bark depth gauge so under-bark diameter can be estimated from over-bark diameter. Generally two measurements of bark thickness at opposite points on the stem will be sufficient to estimate average bark thickness. However, if the measurements differ by > 0.2 cm on stems < 10 cm diameter, or > 2% on larger stems then another two measurements should be made (total four equally spaced measurements).

The main reasons for measuring stem diameter under- and over-bark are for application to estimating the dry weight of dead trees and in some cases for the estimation of bark.

2.2.2 TREE HEIGHT

Tree height should be estimated before trees are felled. This is because assessment of tree height in inventory will always be done on standing trees. If ground-based inventory is expected then tree height should be measured by ground-based methods. The height (stem length) after felling should also be recorded to enable potential application of other inventory techniques (eg. remote-sensing).

Height sticks may be used for an accurate estimate of tree height though their use is generally restricted

to small or medium-sized trees. Other ground-based methods for estimating tree height involve trigonometry. A classical system includes a tape to measure distance from an observer to the tree and a clinometer to measure angles from the observer's eye to the tree base and tree top. Modern digital hypsometers simplify the process. Tree height should be measured at right angles to any lean. Preferably two estimates of tree height should be made on opposite sides of a tree to confirm estimates within the precision of the instrument. Where trees are large, errors in measuring height can be considerable using ground–based methods. This is due to difficulty in observing the top of the tree due to obstruction from other vegetation or the shape of the crown. For example, many eucalypts have a more open rounded canopy than conifers making it difficult to identify the true top of the tree.

2.2.3 CROWN DIMENSIONS

Amongst the crown dimensions that may serve as useful predictor variables are crown diameter, crown area and crown depth. Generally crown dimensions will be most useful in woodlands or more open forest communities rather than closed forests where 'crown closure' restricts further crown development.

Crown diameter may be estimated from the average of two measurements at right angles. If necessary the edges of the crown can be "plumbed" to the ground level to allow measurement. An optical device that provides a vertical line of sight (crownometer) can be used or a carpenter's plumb line will also be effective.

A simple estimate of crown cover (crown area) may be obtained from crown diameters (assuming circular or elliptical shape). More precise estimates are obtained by point sampling (Figure 2.1). For example, use a crownometer to record presence or absence of crown (projected foliage cover) at grid intersections. This may be the only option for accurate estimation of crown area of irregularly shaped crowns typical of large eucalypts.

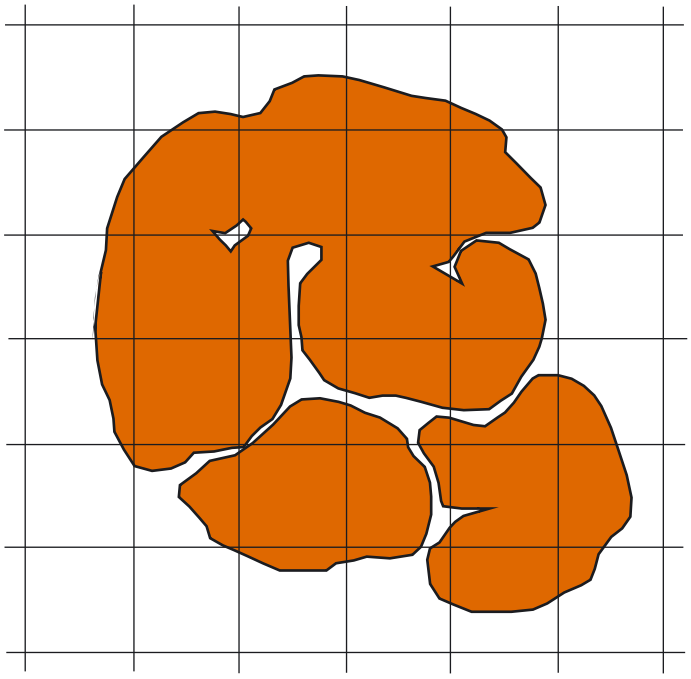


Figure 2.1 Estimation of crown area by point sampling.

To estimate crown area multiply the number of "hits" (grid intersections with projected crown) by the grid cell area.

If estimation of crown area by remote sensing is anticipated then an estimate of crown area on destructive sampling of trees would be relevant.

Height to the base of the crown should also be measured using the same ground-based methods as for tree height. Height to crown base in itself may be a useful predictor variable for crown biomass, particularly in timber production forests subject to pruning. Crown depth is derived as the difference between tree height and height to crown base.

2.2.4 DEAD TREES

For dead trees and stags an estimate should be made of the degree of degradation (eg. Eyre *et al*. 2000) and estimates made of the proportion remaining of each of the following:

- Leaves;
- Branch bark;
- Branch wood;
- Stem bark; and
- Stem wood.

2.3 COMPLETE HARVEST METHOD

The complete harvest method is so-called because all above-ground material in destructive sample trees is collected and weighed. It is the most accurate method for determining above-ground biomass and hence is generally the preferred method. Often, complete harvest will be also be quicker than alternative partial harvest methods.

Circumstances where complete harvest may not be the preferred method or not possible include:

1. Very large sample trees with restricted access so that weighing the entire tree is difficult;
2. In situations where partial sampling is quicker than complete harvest it may be more efficient to sample more trees by partial harvest, though with less accuracy for individual sample trees; and
3. If felling of sample trees is not allowed then partial-harvest methods will be necessary.

In these circumstances partial harvest methods outlined under partial harvest methods can be used.

If detailed partitioning of components of above-ground biomass is required then partial-harvest methods may also be relevant. In particular this applies to leaf biomass because of the large amount of work involved in removing leaves from the complete crown of all but small trees or shrubs. If a separate estimate of leaf biomass is required it may be best to combine the complete harvest method with another method. Thus complete harvest can be used to obtain an accurate estimate of total crown biomass while partial harvest can be used to estimate the proportions of branchwood, twigs and foliage.

2.3.1 SELECTION OF COMPONENTS

The above-ground portion of a tree is divided into components for separate fresh- and dry-weight determination. Various considerations will determine selection of components:

1. **Moisture content.** Material should be grouped by moisture content and therefore dry-weight:fresh-weight (DW:FW) ratio. For example, dead branches will have lower moisture content (higher DW:FW ratio) than live branches and so live and dead branches should be separated;
2. **Carbon concentration.** Material should be grouped by carbon content. Carbon concentration of foliage will differ from that of wood. Thus, in young trees where the proportion of foliage weight compared to the total can be high separate estimates of foliage and woody material should be made;

3. **Practicality**. It may be more efficient to separate some material to facilitate the taking of representative samples as will be required for determining DW:FW ratio or carbon content. Thus, if a species has large branches, it may be best to partition the branches by diameter. For example, all branch material with a diameter > 5 cm could be one component and smaller branch material, including twigs and leaves, another component; and

4. **Interest**. Selection of components can also be based on those of interest, eg. a separate estimate of leaf biomass may be of interest as well as an estimate of whole crown biomass (leaves, twigs and branches included).

As a minimum the following components should be identified:

- Stem;
- Crown;
- Dead attached material; and
- Dead detached material (if present).

Stem

The border between stem and root should be made at ground (mineral earth) level.

Division between stem and branches can be difficult. In general the stem is defined as the thickest shoot, including the trunk, leading to the top of the crown. However, double- or multi-stem trees may also be recognised. In these cases two or more shoots will have grown to a similar height with neither dominant.

Sometimes the data will be used to calculate expansion factors so that whole tree biomass can be estimated from total or merchantable stem volumes derived from inventory. It then becomes necessary to have a definition of stem consistent with that used in the inventory. In lightly branched species, merchantable volume is usually calculated from a nominated stump height to a minimum upper stem diameter (eg. 10 cm). In more heavily branched species merchantable volume is defined by the point of insertion of the first major branch which would interfere with the recovery of sawn timber. The remaining proportion can be regarded as an additional component of non-merchantable stem but often this is included with the crown. Division of the stem into sub-components, eg. wood and bark is optional[1] unless merchantable volume for the species is defined under bark. Such partitioning is best done on disc sub-samples.

Crown

The crown should include all live material attached to the stem. This will include branches, twigs, leaves and, if present, flowers and fruit. Any other plants supported by the branches, such as vines and epiphytes, should also be included. The cut to separate branches from the stem should be made flush with the stem.

Separation into sub-components is optional. Separation of large- and small-branch material is likely to be based on practical sub-sampling considerations. Also, as previously mentioned, a separate estimate of leaf biomass may be of interest.

Dead attached material

Dead attached material (DAM) is most likely to consist of dead branches but may include other material such as dead stem segments and flaking bark. Only include material still attached to the stem in this category. Heartwood within live stem or live branches should be included within the 'stem' and 'crown' categories.

If DAM is a large portion of the dry weight of sample trees and dry weight changes over time then it may be necessary to recognise different categories

[1] Although wood and bark may have differing moisture contents and therefore differing DW:FW ratios disc samples taken accross a stem will have bark:wood ratios consistent with that of the whole stem and therefore *DW:FW* ratios representative of the whole stem.

of DAM. For example, these may be (i) dead branches with leaves and bark still attached, (ii) sound dead branches without bark and (iii) rotten dead branches.

Note that dead trees are a special case. Both stem and attached branches of dead trees should be included in the DAM category.

Dead detached material

Some trees will have a substantial amount of dead detached material (DDM) held above-ground in the crown or stem forks. This may include fallen branches and leaves as well as shed bark.

2.3.2 FRESH WEIGHT DETERMINATION

Fresh weight of components should be obtained in the field. It is not essential that material be weighed immediately a tree is felled, or even the same day a tree is felled. Thus the term 'fresh weight' is applied loosely to indicate 'weight recorded in the field'. However, once measurements and sub-sampling for DW:FW ratio commence on a component of a sample tree the work should be complete in as little time as possible. This is to minimise any drying (weight loss) over the period while measurements are made.

There are a variety of acceptable options for weighing whole components. The classic system includes a tripod, spring balance, block-and-tackle, and cradle for the load. With an efficient block-and-tackle one person can comfortably lift loads of several hundred kilograms.

A bipod with stability provided by a guy rope (Figure 2.2) may be an improvement on the tripod as there is greater access for loading the cradle. This system can be lightweight and easily manoeuvred.

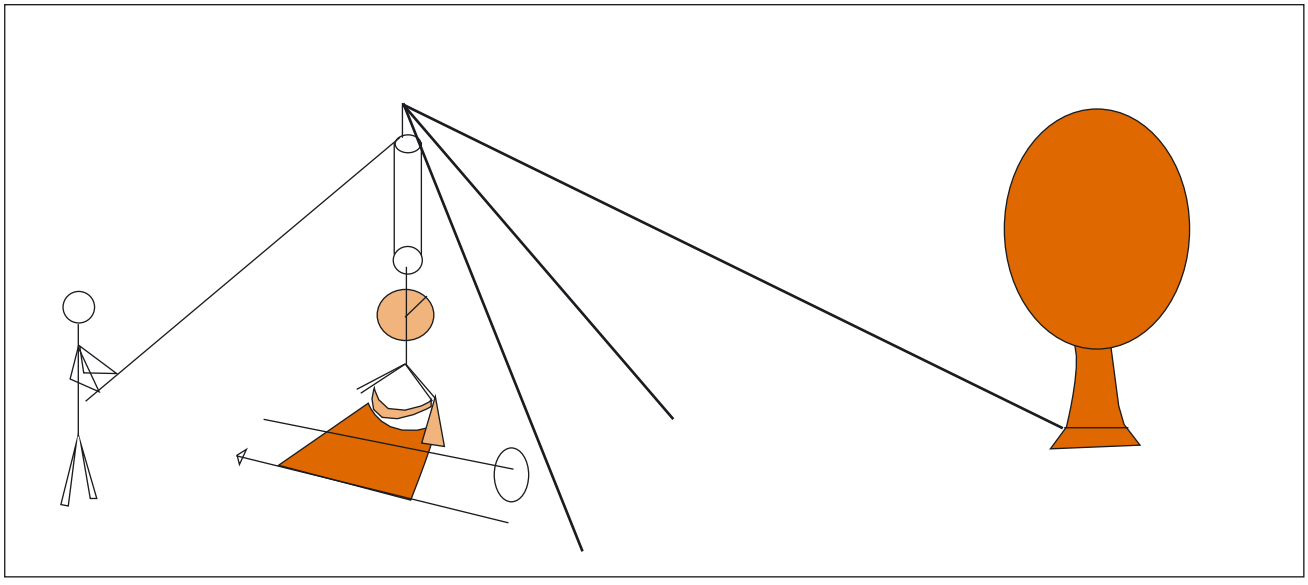


Figure 2.2 Bipod system for obtaining fresh weights of tree components.

The guy rope may be attached to a tree or a vehicle. Use of a block-and-tackle reduces the effort required to lift heavy loads.

Digital scales and load sensors are also available with small or large capacities (up to 10 tonnes or more). These may be either top loading, such as cattle scales and small weigh-bridges, or designed for suspended loads. Using a load cell suspended from a excavator arm allows weights of up to 5,000 kg to weighed quickly and accurately on rough terrain. Thus direct weights of even the largest trees can be directly obtained. For example, a 75 cm DBH tree will weigh roughly 9 tonnes fresh weight. By weighing the trunk and canopy separately a direct estimate of tree fresh weight can be obtained.

Any machines on-site may be used to hoist loads for weighing. If logs are being recovered from the sample tree then logging machinery may be useful. Some tree-harvesters even have built-in weighing equipment designed to weigh logs. Otherwise, earth-moving machines such as back-hoes being used for root sampling may be utilised.

Generally the balance should be accurate to < 1% of the load. Depending on the equipment used this may necessitate the use of several balances, eg. balances with capacities of 10 kg, 100 kg and 1,000 kg for fresh weight determination of large trees. If a large capacity balance is not used then some components of large trees (stem and crown components at least) will have to be weighed in several lots.

Similarly, it may be convenient to have more than one cradle for varying loads. This could be a cradle made from reinforced heavyweight tarpaulin for bulk loads of heavy branches or large stem pieces and lightweight cradle for small loads. Slings may be most suitable for medium and large stems.

Material removed in saw cuts should be considered. Calculate this initially and if the volume of saw cuts is more than 0.5% of a component then the cuts should be accounted for. As an indication, if a stem is cut into 2 m lengths for weighing and the chainsaw cuts are 1 cm wide, then cuts will be approximately 0.5% of the volume. One way of accounting for the cuts is to estimate the cumulative volumes of cuts (∑(cross-section areas x saw cut width)) and multiply by the fresh (green) density of the material. Green density can be estimated from the volumes and weights of sample pieces. (Green density = green weight / green volume.)

2.3.3 DRY WEIGHT CONTENT

Basic steps in dry weight determination are:

1. Representative sub-sample(s) of each component of destructive sample trees are weighed 'fresh' (i.e. at the same time as the entire component is weighed in the field).
2. The sub-samples are oven-dried at a specified temperature (here 70°C is used) to constant weight.
3. Dry weight (biomass) of a component is estimated as the product of the fresh weight of a component and the DW:FW ratio of the sample.

Sufficient material should be sampled to be representative of the component but large samples will take longer to dry. Generally samples of around 300 g – 1000 g will be suitable.

Take samples from the main components as outlined in the following sections.

Stem

Three acceptable methods are outlined.

Method S1: This can be applied if initial sampling indicates there is no significant trend in moisture content along the length of stems. In this case visually divide the stem into three sections (height intervals) of approximately equal volume and take a disc sample from the middle (by volume) of each section. Location of sampling heights by visual estimation is acceptable. As a guide to estimating the middle one-third of stems the centroid (centre of mass) of stems is usually located at around 0.2-0.3 of tree height (Wood *et al*. 1990; Bi 1999). One way of determining the precise location of the centroid is to balance a stem on a suitable pivot such as another log at right angles. The balance point will be the centroid.

If there is more than one stem at a sampling height then take a disc from each stem at that height. All stem discs from sample trees should be of equal thickness. They can be bulked to form a single sample for weighing. Alternatively, if discs are

weighed separately, eg. to indicate if there is a trend in moisture content along the length of a stem, then sum the weights of all discs to derive one estimate of DW:FW of the stem

$$FW{:}DW = \frac{\sum_{i=1}^{n} DW_i}{\sum_{i=1}^{n} FW_i} \qquad (2.2)$$

where DW_i and FW_i are the dry weight and fresh weight of the ith disc.

If there is no apparent trend in moisture content along the length of a stem then precise location of sampling discs is not critical. Thus, if logs are being taken from a sample tree, take disc samples from the nearest locations to those indicated above such that log recovery is not compromised. However, do not take a disc from the end of a log or other stem section unless it is done at the time the stem was initially sectioned. This is because ends will dry rapidly.

Method S2: This should be applied if moisture content is found to vary systematically along the length of stems or sampling to test for such systematic variation has not been done. The stem is cut into at least three billets of a convenient size for weighing in the field. A disc sample should be taken between each successive billet and at the base and top of the stem section. All discs should be of equal thickness. Discs should be cut and weighed soon after falling to avoid drying of cut ends.

Sum the weights of a billet's two discs to estimate DW:FW ratio for the billet (Equation 2.2). The dry weight of each billet should be estimated separately by multiplying billet fresh weight by DW:FW ratio.

Method S3: This should be applied if estimates of stem volume and stem density are required to develop relationships whereby biomass can be estimated from inventory data for stem volume, stem density and biomass expansion factors (BEF) (Refer Appendix A3.6). Fresh weight of the stem is determined as above. Volume is measured using the techniques given in Section 2.4.1. Samples are taken to determine DW:FW ratio according to Method S1, Method S2 or at locations determined by importance sampling (IS) based on cumulative volume of the stem (see Section 2.4.1). Stem biomass is estimated in the usual way, then stem density is estimated by dividing stem biomass by stem volume. Note that this will be biomass density (based on a drying temperature of 70°C), not the usual basic density for wood (based on a drying temperature of 105°C).

Note that the measure of stem volume obtained from the IS method will be total stem volume over-bark. If the species is a commercial timber species for which stem volume data are available then it is likely that it will be in the form of merchantable volume under-bark or total volume under-bark. In these cases corresponding estimates of stem volume under bark should also be made on the stems of sample trees to facilitate calculation of BEFs.

Crown

Method C1: To be applied if initial sampling indicates no systematic variation in moisture content between branches within the crown, eg. compare moisture content of branches from the top and bottom of the crown.

Divide the crown into ≥ 3 sections of approximately equal biomass for sampling. The sections could be separated by height, eg. top, middle and bottom sections of the crown. Another possibility would be to use vertical divisions between sections, eg. to divide the crown into quarters.

If the crown is being partitioned into large branch material and small branch material (including twigs and leaves) based on a diameter limit then cut the branches in each crown section at that diameter limit. This will provide two sets of material for each crown section. The fresh weight of each should then be determined.

The large branch material should be sub-sampled for DW:FW ratio as for stems (Method S1, S2 or S3).

The smaller branches (or complete branches if large and small classes have not been separated) are treated as below.

Visually select a sample branch from each section. Each sample branch should be representative of its section, i.e. similar proportions of leaf, twig, and larger branch material. If branches are small and numerous, take sufficient branches so that the sample weight is approximately five percent by weight of the total crown section. These can be bulked for weighing and drying or sample branches handled separately and an average DW:FW ratio calculated. If sample branches are handled separately and estimate of error bounds on the estimate of total crown can be made.

If a sample branch is too large for drying, a sub-sample is required. This is taken after determining the total fresh weight of the sample branch. The sub-sample should comprise an equal proportion of leaf, twig and larger branch material to that in the whole sample branch. Take a defined proportion of each part, eg. if a branchlet with 1/10th of the leaf and twigs is selected than also take 1/10th of the larger branch material.

Method C2: To be applied if 1) systematic variation in moisture content between branches in the crown is apparent, or 2) a separate estimate of leaf biomass is required. The basic approach is to first stratify the branches into groups with similar moisture content and/or proportions of foliage. Strata may then be determined by factors such as basal branch diameter, branch length or position of branch within the crown. The total fresh weight of each stratum is measured and a sample of branches is taken from each to determine DW: FW ratios. Proportions of branch-wood: foliage are also measured if necessary (see Method L1). These proportions are then applied to the fresh weight measurements to provide estimates of dry weight.

Method C3: Sample branches can also be chosen with probability proportional to size (PPS sampling). List sampling or random branch sampling (RBS) may be applied. In list sampling a complete enumeration of some aspect of branch size is required. Usually branch diameter is measured but length measures (L) can also be included into a composite variable such as D^2L. Lists of branch size and associated cumulative size are then prepared. A random number between 0 and 1 is selected and multiplied by the largest cumulative size. A branch is chosen if this number is less than or equal to its cumulative size and greater than the previous branch's cumulative size (Shivers and Borders 1996). Alternatively the list can be used to select boundaries for strata from which simple random samples of branches may be taken (see Appendix A3.2.2). At least three sample branches should be taken to determine DW: FW ratios. Proportions of branchwood: foliage are also measured if necessary (see Method L1). These proportions are then applied to the fresh weight measurements of the total crown to provide estimates of dry weight. Note that in the case of list sampling it is feasible to make a regression based estimate of branchwoood: foliage ratio (see Method L2).

A RBS procedure in which selection probability is proportional to branch diameter raised to the power 2.5 ($d^{2.5}$) is described in Section 2.4.2. RBS is less suited to trees with strong apical dominance, such as many conifers and close-spaced eucalypts, because the stem is included in the sampling pathway and there is often a strong vertical gradient in crown characteristics such as branch size and proportion of foliage.

Leaf biomass

If a separate estimate of leaf mass is required then this can be estimated as a proportion of the total crown biomass. Estimate crown biomass by the complete harvest method as previously described. Then apply either of the following methods.

Method L1: Select ≥ 3 sample branches from the crown (or from the 'small branch material' component if branches are partitioned at a diameter limit to form two crown components). These should

be the sample branches selected for dry weight determination (Method C2 above) increasing the number of sample branches, if necessary, to achieve a minimum 5% by weight of the component sampled.

Partition the sample branches into leaf and non-leaf material. Note that 'leaves' should include the petioles. If required the non-leaf material can be further partitioned, eg. into twig and larger branch material.

Estimate leaf dry weight (DW_L) and total branch dry weight (DW_{TBR}) of the sample branches. Note that 'total branch' includes all material attached to a branch, eg. twigs, leaves, fruit, epiphytes. DW_{TBR} should be calculated as the sum of the dry weights of leaf (DW_L) and non-leaf material.

Calculate the average DW_L:DW_{TBR} ratio for the sample branches. Apply this ratio to the estimate of crown biomass (B_{CR}) to estimate leaf biomass (B_L) in a destructive sample tree:

$$B_L = \frac{DW_L}{DW_{TBR}} B_{CR} \qquad (2.3)$$

Method L2: This method uses branch regression techniques to estimate leaf biomass as a proportion of crown biomass. In a simulation study Snowdon (1986) showed that such an approach to estimating branchwood as a component of crown biomass doubled accuracy compared to the branch regression approach alone.

Use the regression method to estimate the DW_L and DW_{TBR} of each branch on a tree. This will require the measurement of the appropriate predictor variables, eg. branch diameter, branch length etc., on each branch of the sample tree. Estimate leaf biomass of a sample tree (B_L) as:

$$B_L = \frac{\sum DW_L}{\sum DW_{TBR}} B_{CR} \qquad (2.4)$$

where $\sum DW_L$ and $\sum DW_{TBR}$ are sums of regression estimates of leaf dry weight and total branch dry weight of all branches and B_c is the estimate of crown biomass derived from the complete harvest method.

Dead attached material (DAM)

Take a sample with the same ratio of DAM in the form of branches, stem, or other material as on the whole sample tree. This representative sample can be sub-sampled if necessary to derive a sample of suitable size.

If a whole sample tree is dead or there is a large proportion of DAM then different categories should be identified and sampled separately. For example, if a sample tree has many dead branches in varying stages of decay it will be necessary to identify categories such as i) recently dead, ii) no bark but sound and iii) rotting.

Dead detached material (DDM)

If DDM is present in the destructive sample tree then take a sample representative of the material present.

2.3.4 WEIGHING AND HANDLING SAMPLES

Weighing

It is essential that weighing of a component for fresh weight and sampling from that component to estimate the DW: FW ratio be done at the same time. This is because live material will lose moisture rapidly once the a sample tree is cut up and dead material may gain or lose moisture, eg. rapid gains in the event of rain.

Samples should be weighed immediately they are taken in the field. A battery powered digital balance with capacity of 1000 g and accuracy of ± 1 g is suitable for this.

After field-weighing the samples should be kept in cool dry conditions to reduce biomass loss from respiration. Keep samples in cooler boxes and in shaded or air-conditioned vehicles while in the field.

Place samples in cool (2 – 4°C for temperate species, 12 –14°C for tropical species) storage immediately on return from the field unless they are to be oven-dried immediately. This can extend storage life to 2 weeks if required with minimal dry weight loss (Forrest 1968).

Oven-drying

Samples should be oven-dried to constant weight at 70°C in a fan-forced oven. Such temperatures do not remove all moisture from plant tissues but higher temperatures (> 80°C) may cause a loss of dry weight due to decomposition of some organic compounds and volatilisation of some vegetative oils (Reuter *et al*. 1997). For large wood samples, eg. stem discs, drying times can extend to weeks.

Carbon concentration

An estimate of carbon concentration of each component sampled should be obtained for each 'vegetation type' x region combination. This should be based initially on ≥ 5 samples from each component, sampling each of the major species in a 'vegetation type'. Depending on the variability found more samples may be required (Gifford 2000). Take and analyse sufficient samples to estimate mean carbon concentration of a component to ± 2 percentage points, eg. 50±2%, with 95% confidence.

Samples obtained for dry weight determination are suitable for carbon concentration analysis. Samples should be ground to a particle size < 0.5 mm and homogenised. Sub-sampling will be necessary as only a small amount (~ 2 g) will generally be required for analysis. It is important that the samples are re-dried to 70°C (not a higher temperature) and submitted for carbon analysis at this moisture content.

The ground powder sub-samples should be analysed for total carbon concentration by a combustion furnace method (Rayment and Higginson, 1992: Method 6B2 or 6B3). Here, a small sub-sample burnt in pure oxygen at high temperature to convert any carbon to carbon dioxide (CO_2). The CO_2 is carried through to a detector in a stream of pure helium, other oxides (nitrogen, sulphur, etc.) being removed from the gas stream. The concentration of CO_2 is measured by non-dispersive infrared absorption. Alternative methods for carbon analysis include gas chromatography and mass spectrometry (Gifford 2000). With the combustion furnace method samples may also be conveniently analysed for total nitrogen concentration by the combustion furnace method at the same time as analysis for total carbon concentration. Additional analyses for other nutrients can be considered to increase the value of the data.

2.3.5 CALCULATIONS

1. Biomass of each component (B_c) of a destructive sample tree is estimated as the product of the fresh weight of a component (FW_c) and the ratio of 'dry weight : fresh weight' of the sample (DW_s/FW_s):

$$B_c = FW_c \frac{DW_s}{FW_s} \qquad (2.5)$$

2. Carbon content of each component (C_c) is estimated as the product of estimated biomass of the component and the relevant estimated mean carbon concentration ([C]):

$$C_c = B_c * [C] \qquad (2.6)$$

3. Total carbon content in the above-ground 'biomass' pool is estimated as the sum of components:

$$C_{AG} = \sum_{i=1}^{n} C_{ci} \qquad (2.7)$$

where

C_{AG} = carbon content above-ground; and

C_{ci} = carbon content of the *i*th component.

Error estimates

Estimates of component biomass should be reported in company with the errors involved in arriving at the estimate. With complete harvest methods there are two main sources of error. The first, measurement error, is determined by the accuracy of instruments used to measure fresh and dry weights. The second can be attributed to sub sampling errors when samples are taken to estimate DW: FW ratios. Several samples should be used to estimate DW: FW ratios so that their standard deviation can be used to assess this error component.

2.4 PARTIAL HARVEST METHODS

Tree size, accessibility and ability to fell the tree may limit the use of the complete harvest method. Under these conditions alternate approaches are required to determine above-ground biomass of woody vegetation. Partial sampling methods involve indirect measurement and/or subsampling of the tree to estimate the biomass of the tree. These methods do not give as accurate measure of tree biomass as the complete harvesting methods outlined above. For this reason it is strongly recommended that the methods outlined below be used *only* when complete harvest methods can not be used. This is particularly so if the tree biomass data are being used to develop allometric relationships. Errors introduced in this primary data set will be propagated when these relationships are used to provide estimates of biomass across landscapes. However, the methods outlined below are very useful in estimating different components and give good levels of accuracy when used in conjunction with complete harvest methods.

Estimation of tree biomass using partial harvesting methods essentially involves two distinct steps:

1. Estimation of stem and large branch biomass from volume measurement combined with estimation of density from disc or core samples; and
2. Estimation of foliage and smaller branch biomass from regression sampling or by sub-sampling methods.

2.4.1 ESTIMATING THE BIOMASS OF STEM AND LARGE BRANCHES USING VOLUME AND DENSITY

Measurement of stem and branch volume

Stem and branch volume can be calculated from measurements of diameter and length. For commercial tree species volume functions may be used to calculate the volume of the main stem (eg. Bi and Hamilton 1998). However, estimates based on such generic equations will be less accurate than estimates based on direct measurement and calculations using geometric models outlined in Table 2.1. This approach can be applied to both stems and large branches. The shape of stem/branches and the number of measurements that can be taken will determine the best models to be used.

Application of classical dendrometry to approximate stem volume requires stem diameter measurements at measured intervals along the stem. Diameters are commonly measured midway between branch nodes in order to avoid stem swelling that occurs near nodes. Alternatively diameter measurements can be taken at set distances (eg. 10 cm) above and below nodes. This is method is also used whenever there is a sharp change in taper along the stem. Volume can then be calculated by assuming the conic or paraboloid frustrum equation in the upper part of the stem, cylindrical or paraboloid frustum equation in the middle section of the stem, and a neiloid frustum in the rapid tapering base of the tree (Table 2.1). The exact function used will depend on the measurements taken. Total volume is estimated by summing the total volume of all segments. Further guidance on the application of these equations is available from Brack and Wood (1998).

Branch volume can also be estimated using the methods outlined above, particularly in large trees where branches can approach the size of small trees. The branch size to which this method can be applied to will be determined by branch diameter, branching pattern and the practicality of applying alternative sub-sampling methods outlined below. These factors will also determine the small end

diameter limit beyond which sub-sampling methodologies should be used to estimate the remaining branch and foliage biomass. Branches are more irregular structures than stems hence branches will need to be measured in a larger number of segments. The volume of each segment is then calculated separately and each segment summed to give branch volume. The number of segments required will be determined by the uniformity of the stem or branch. The following criteria is suggested for dividing the stems or branches into segments (Pillsbury and Kirkey 1984):

1. Segments are defined if abrupt changes in taper occur;
2. Segments are defined as the distance from fork to fork; and
3. Where a branch has a distinct abrupt change in direction, segments are defined to give straight branch lengths.

The smaller the segments used the less will be the error introduced by making incorrect assumptions about branch shape.

Table 2.1 Geometric models useful in the calculation of portions of a stem volume (Telewski and Lynch 1991). Note area can be substituted with diameter where A = d^2 x 0.7854

	Geometric shape	Formula name	Formulae
(1)	Cylinder		$L \times A_b$
(2)	Paraboloid frustum	Huber's	$L \times A_m$
(3)		Smalian's	$L\left[\frac{A_b + A_u}{2}\right]$
(4)	Cone		$\frac{H \times A_b}{3}$
(5)	Cone frustum		$\frac{L(A_b + \sqrt{A_b \times A_u} + A_u)}{3}$
(6)	Frustum of paraboloid, cone, or neiloid	Newton-Simpson	$\frac{L\,(A_b + 4A_m + A_u)}{6}$

H = height;

L = length of frustum;

A_b = area of the base;

A_u = area of the frustum's upper cross section; and

A_m = area of the mid-length section.

Measurement of stem and branch density

Density of the stem or branch is required to convert volume measurements to mass. This raises the question of where to sample. If density does not systematically vary within the component (stem or branch) then a simple random sample can be taken. However, variations in density are often observed with tree height, although the trends are not consistent in eucalypts (Downes and Raymond 1997). Where systematic variation occurs, a systematic or an importance sampling strategy should be used. In the importance sampling method there is a greater probability that samples will be taken from the region which contributes greatest to the size of the object being sampled, i.e. samples are taken with probability in proportion to estimated size. This produces unbiased estimates (Valentine *et al*. 1984).

Two main methods are used to measure density these being discs or cores. Discs require destructive harvesting of the tree and are therefore restricted to situations in which the tree has been felled. However, where possible discs should be taken as these provide a representative sample of the density across the stem or branch being sampled. The

methods for measuring density have been outlined in Ilic *et al*. (2000). Density is estimated on the basis of dry weight per unit green volume. Volume is estimated from diameter and thickness measurements of fresh-weight samples. Sample weight is determined after oven drying. Samples should be dried at 70°C to maintain consistency with the drying temperatures of other plant parts. If dried at 101-105°C then dry weight will be 2 – 4% less and should be corrected to the 70°C standard.

Bark is usually included in the density of the entire disc or core, while-over bark volume is estimated. However, separate estimates can be made of wood and bark volume, density and biomass. In this case both over and under-bark measurements are made and separate dry weights are determined for wood and bark. Wood volume is estimated from under-bark diameter and disc thickness while bark volume is estimated using thickness and both diameter measurements.

The use of published values of density (eg. Bootle 1983) is not recommended. Density will vary with age and growth environment thus sampling of the tree being measured is required. In addition, over bark volume is usually measured in partial sampling methods. Published density values usually refer to wood density only. Consequently, if over bark volume has been calculated then discs should also include bark.

Cores can be taken from standing trees. However, cores over sample the center of the stem and under sample the outer rings and bark (Figure 2.3). Hence if density is measured using a coring method then a weighted density value is required to convert stem volume to mass. The core density values should be broken into suitable increments (this may be arbitrary divisions eg. 20 mm increments, or be based on expected changes across the stem eg. sapwood and heartwood) and each increment weighted according to the calculated cross-sectional area.

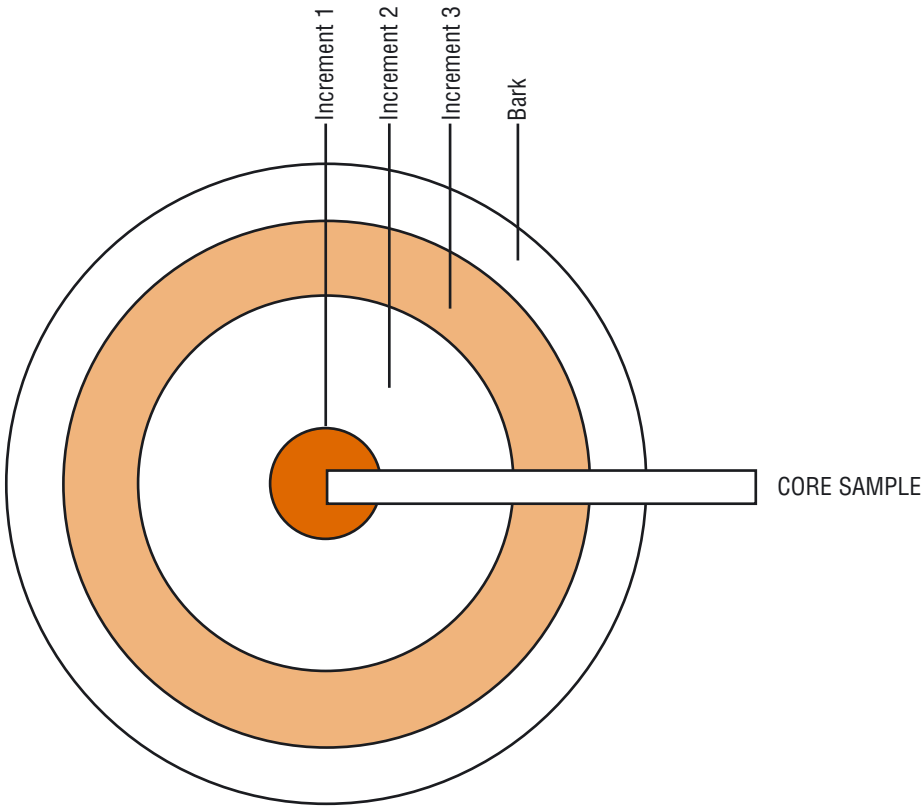


Figure 2.3 Stem cross-section showing core sampling positioning. Density measured by coring requires increments to be weighted to reflect the cross-sectional areas of the increments.

Estimating biomass

Method SB1: Systematic sampling. This should be applied if wood density is found to vary systematically along the length of stems or branches. The stem or branch is measured for volume estimation as outlined above. The locations for diameter measurements are noted or marked. The stem or branch is divided into a convenient number of billets the ends of which are defined by previously marked locations for diameter measurements. (Note not all measurement locations need to mark the end of billets). A disc sample is taken between each successive billet and at the base and top of the first and last billets respectively. Discs should be cut and weighed soon after falling or branch removal to avoid drying of cut ends. The dry weight of each billet is estimated by multiplying billet volume by the cross-sectional-area weighted density (Equation 2.2) of its two adjacent discs.

It is convenient to use this method when separate estimates are required for bark and wood biomass and when data is required for biomass expansion factors based on under-bark volume. Under-bark diameters are measured on each disc so that total billet volume can be partitioned into wood and bark volumes. Separate estimates are made of wood and bark densities. These are then applied to the volume measurements to obtain estimates of wood and bark biomass.

Method SB2: Importance sampling. Below we outline how importance sampling can be used to determine the sampling position for density or dry weight content of the stem or branch pools. Importance sampling requires an estimation of the size, usually volume, of the stem to determine the sampling point. Stem volume can be estimated either using an appropriate volume function or by techniques outlined above. The following calculations are required to determine the location of discs/cores to be taken. At least two sampling points are recommended.

Volume of each section and cumulative volume of the whole stem are calculated using the methods outlined above (Table 2.2).

Table 2.2 Calculation of cumulative stem volume.

Height of stem (m)	Diameter over bark (cm)	Sectional area (m^2)	Cumulative volume[1] (m^3)
0			
2	51.5	0.2083	0.4166
4	40.0	0.1257	0.6679
6	33.7	0.0892	0.8463
8	31.0	0.0755	0.9973
10	29.8	0.0697	1.1368
12	27.4	0.0590	1.2547
14	25.4	0.0507	1.3561
14.5	26.5	0.0552	1.3836

[1]Calculated using equation 1 in Table 2.1

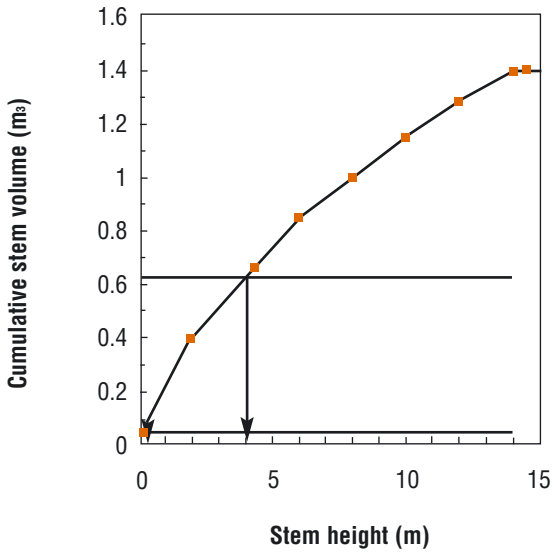
A random number between 0 and 1 is generated and multiplied by the total cumulative volume. The resulting value gives the position within the total cumulative volume from which the disc is to be sampled (Table 2.3).

Table 2.3 Determination of the location of the sampling point based on the volume of the stem.

Random number	Total stem volume (m^3)	Sampling position (m^3)	Tree height* (m)
0.03316	1.3836	0.045881	0.25
0.44539	1.3836	0.616259	3.60

*The height at which to take the sample is determined graphically (Figure 2.4). This procedure is repeated to obtain two or more sampling positions.

Figure 2.4 Determination of stem sampling height based on cumulative volume. Refer to text and Table 2.3.



Sampling and measurement requirements for density calculations

Stem or branch biomass is calculated by multiplying stem volume by average density. An estimate of error can be based on the different values of density obtained.

2.4.2 ESTIMATION OF BRANCH AND FOLIAGE BIOMASS

The volume method outline above can be used to estimate the biomass of large branches. However, this method requires all branches be measured. If the tree is standing such measurements will be difficult. Below we outline alternative subsampling approaches to calculate biomass of major branches. They are also applicable to calculating biomass of small branches, twigs and foliage.

Regression based estimates

The regression and ratio sampling methods used to estimate tree biomass (Appendix 3), can also be applied to estimate branch (Attiwill 1962; Snowdon 1986) and foliage biomass (Cherry *et al*. 1998). The method depends on the development of relationships between component biomass and a set of independent variables. Branch diameter is frequently used as the independent variable in regression equations (branch diameter is measured above the basal swell where the branch joins the stem). Strong relationships can be expected between

branch diameter and total branch or branchwood biomass. Foliage is not as strongly related to branch diameter. This is partly due to the dynamic nature of the foliage. Seasonal changes in both evergreen and deciduous species, fire, water and nutrient availability and competition for light (both within and between trees) will affect the retention of foliage on a branch. Thus it is important to clearly outline the conditions under which the regression relationship was developed if it is to be used beyond the population of trees from which it was developed.

To account for more of the variation in foliage biomass other branch and tree related descriptors should be included in regression models. These may include crown position or height to branch insertion in the stem, branch length or age, projected crown area and tree size. These descriptors attempt to adjust the branch diameter foliage biomass relationship for within and between crown competition and climatic and site conditions.

The method requires destructive sampling of some branches to develop the relationship between the independent variables and branch biomass. For statistical considerations of sampling design and sample number refer to the development of such relations in Appendix 3. In this section we outline considerations when the regression approach is used to estimate branch and foliage biomass. Again we would like to highlight that when such methods are used alone they do not provide an accurate estimation of branch or foliage biomass. A combination of these methods with complete harvest can result in a doubling of accuracy (see Snowdon 1986 for an example).

The procedure for developing the relationship is outlined below.

1. Stratified sampling techniques are recommended. Branches should be stratified based on diameter and possibly crown position, which can improve estimates (Snowdon 1986) but not in all cases (Cherry *et al*. 1998). If the relationship is to be applied to a number of trees then branches from a number of trees need to be sampled. A general equation can be improved by the inclusion of auxiliary variables such as crown position, branch length, tree size (DBH, height), projected crown area (measure the distance across the crown in two directions at right angles and calculate the projected crown area);

2. Once strata have been defined branch diameters and other independent variables are measured for each branch on the tree. Branch diameters are measured beyond the basal swelling that occurs at the junction with the stem (typically 2 - 10 cm away depending on average branch size, this distance should be standard for all branches sampled);

3. Sample branches should be randomly chosen from each stratum;

4. Branches are cut flush to the stem and fresh weight determined in the field;

5. Depending on the size of the branch, partitioning may be required if moisture or carbon content varies. Branch pools can include large limbs, twigs (current years if discernable or based on a size criteria eg. 1 cm diameter) and foliage. On large branches separating foliage from twigs is time consuming. An alternative approach is to cut twigs at a specified diameter (eg. 1 cm) with the foliage still attached. A sub-sample of the twig plus foliage pool is taken to determine a ratio of foliage to twigs. This ratio can then be applied to the total twigs plus foliage fresh weight. Because of possible variation within the tree a sub-sample from each branch should be taken until a measure of variation is determined;

6. Moisture content is determined on each fresh weight pool. The sampling procedure will be determined by the amount of systematic variation in each of the pools to be sampled. For reasonably homogenous samples such as foliage and twigs a sub-sample (using the quartering technique) will be adequate. If branch moisture content is expected to vary then consideration should be given to using importance sampling techniques when the branch is large (see below). If moisture content does not vary systematically random samples will be adequate;

7. The fresh weight of sub-samples should be measured as soon as possible after the fresh weight of the sample has been measured. This applies particularly to foliage, especially if foliage is damp from dew or mist. Sample handling and drying is as previously described;

8. Dry weight of each pool is then calculated based on the FW: DW. Total branch biomass is the sum of the pools;

9. Using regression methods outlined in Appendix 3, a suitable model is developed for the branch data. As well as branch variables the most appropriate model may also include tree level variables; and

10. Apply regression equation to measured independent variables on each branch to estimate total branch biomass of the tree.

Great care is needed if pre-existing equations are used to predict biomass. Substantial bias can be introduced if the population to which the regression relationship is applied is not closely similar to the population from which the relationship was derived. This is particularly important in the case of foliage. Pre-existing regression methods are commonly used for estimating the foliage weight and leaf area of trees because foliage can then be estimated without the need to take destructive samples. However, care is needed in the construction and use of the relationships due to the dynamic nature of the foliage. For example, relationships developed under well watered conditions will not give good estimations of foliage biomass if trees are water stressed. Estimating foliage biomass is further complicated by the effect of light and nutrients on foliage retention. Shading and poor nutrition will speed up leaf senescence at the bottom of the crown. Thus a branch at the base of the crown may sustain considerably few leaves than a branch of the same size at the top of the tree. For this reason stratification of the crown can be important.

Sub-sampling methods

A number of methods exist which sub-sample the crown to provide estimates of foliage and/or branch biomass. These techniques range in sophistication from simple visual estimates known as the "Adelaide" technique (Andrew *et al*. 1979) through to randomised branch sampling (RBS) methods which select the sub-sample based on selection probabilities proportional to estimated size (Jessen 1955; Gregoire *et al*. 1995).

Method BF1: Adelaide technique. The Adelaide technique was initially developed for rangeland shrubs but has also been applied to trees (O'Grady *et al*. 2000). The technique provides approximate values when applied to trees. It is simple to use and well suited to eucalypts growing in open forest situations due to the natural clumping of foliage in the crown. In addition, the method can be totally ground based with sub-samples being pruned or shot down. The method is summarized below for details refer to Andrew *et al*. (1979).

1. For each tree identify visually a crown unit which is typical of the predominant habit, leaf shape and leaf density (note the same crown unit can also be used for a number of trees);

2. The tree is then scored for the number of equivalent "units". This should be done from a number of angles at least three times and preferably by two people independently (generally it takes 1 – 2 days practice before people produce repeatable results). The number of units is the multiplier;

3. Several typical units are removed from the tree (either by climbing the tree or shooting the branch down). Sub-samples of foliage and twigs are taken to determine DW: FW ratios as above; and

4. Total biomass of foliage and twigs within the crown is estimated by multiplying average unit biomass by the number of units.

Random Branch Sampling

Random branch sampling (RBS) can be used to estimate the biomass of branches and foliage in the crown (Valentine *et al*. 1984). RBS schemes are design unbiased, i.e. the average estimate obtained from all possible samples is identical with the parameter value being estimated; they are efficient in that they utilize auxiliary information to select sample units with unequal probability in such a way that the variance of the estimator is reduced compared to equal probability selection schemes; and they can be easier to implement in the field than equal probability schemes (Gregorie *et al*. 1995).

RBS is a method for selecting sub-samples based on probability proportional to size. The sample is chosen by selecting a path from the bottom of the branch (or tree) to the sampling point in such a way that the segments of the path comprise a probability path for the entire branch (or tree). Starting from the base, a branch at each node is selected based on probability proportional to size. Selection probability is typically based on branch diameter which can be weighted depending on sampling objectives i.e. if foliage or branch biomass is being estimated (Table 2.4). The choice of weighting factors will not affect unbiasedness but will affect precision (Gregoire *et al*. 1995). The probabilities of selection assigned to the branches at each node must sum to one. Technically these probabilities are called conditional probabilities because the selection of a branch at a particular node is conditional upon the sampling path reaching that node. The process is continued until a predetermined end point (eg. branch diameter < 5 cm) is reached and a sample taken.

After dry weight of the sample is determined, an estimate of the component total dry weight is then obtained by multiplying the sample value by an inflation factor. The inflation factor is calculated as the reciprocal of the unconditional probability for choosing the particular sample branch. The unconditional probability is calculated as the product of all the conditional probabilities in the path from the base to the sampling point.

Table 2.4 Methods for calculating selection probabilities

Sampling purpose	Probabilities calculation	Reference
Branch biomass or volume	D^2 x length	Valentine *et al.* 1984
	$D^{2.5}$	Gregoire *et al.* 1995
Foliage biomass	D^2	Valentine *et al.* 1984
Eucalypt branch biomass	$D^{2.5}$	Brack and Keith pers. comm.

Method BF2: RBS method for < 5 cm large end diameter branchlets and foliage. In the example, selection probabilities are based on $DOB^{2.5}$. Note that superscript letters relate to the worked example in Table 2.5.

1. Starting at the base of the tree the 1st node where the first live branch intersects the stem is located;
2. Diameter of the stem and each branch at the node is measured, starting with branch 1 as the most vertical (position as if the tree was standing) and working clock-wise[A]. Dead branches are ignored;
3. $Diameter^{2.5}$ is calculated for each branch[B] and a column of cumulative values for all the branches at one node calculated[C] ;
4. A random number between 0 and 1 is generated[D] and multiplied by the total cumulative $DOB^{2.5}$ of the node[C];
5. The branch selected is that with the cumulative value equal or nearest above the value derived at the previous step (eg. branch 2 at the 4th node, see Table 2.5);
6. If DOB^2 x length is to be used in probability calculations (Table 2.4) or if biomass is to be estimated by importance sampling (see Method BF3 below) then the length of section along the branch from the current node to the next node (or to the tip of the branch if the current node is the last node) is measured. This can be used to calculate volume of the branch section [F];
7. Measurement of nodes and selection of branches continues until the selected branch has a diameter ≤ 5 cm;
8. The 5 cm or less branch is cut and the length from the fork to the tip of the branch measured;
9. Foliage on the sub-sample is removed and if appropriate the woody material is partitioned into differing size twigs and branchlets. Fresh weights of these pools are measured and a sub-sample taken to determine moisture content. These are used to calculate dry weight of branchlet, twig and foliage pools;
10. Calculate the conditional and unconditional inflation factors for the path. At each node the conditional probability for selecting the chosen branch is calculated by dividing $DOB^{2.5}$ of the chosen branch by the cumulative sum of $DOB^{2.5}$ for all branches at the node. The conditional inflation factor for the selected branch is the reciprocal of this probability. The unconditional inflation factor for the sample is calculated as the product of all the conditional inflation factors in the pathway; and
11. The dry weight of the sub-sample is multiplied by the unconditional inflation factor to give a total tree estimate.

The procedure is repeated five times thus giving five estimates of crown biomass. The average value is taken as the final estimate of biomass while the standard deviation between the individual estimates provides a measure of the reliability of the final estimate. Variation between fresh weight of individual sub-samples can be used as a guide to determine if sufficient sub-samples have been taken to estimate components of crown biomass within prescribed limits of error. The same pathway and branch may be selected more than once.

In practice small epicormic branches or spur shoots are ignored when selecting the pathway. To avoid bias when a small branch is ignored in path selection, its characteristics of interest (eg. foliage) should be treated as part of the characteristic of the branch to which it is attached. In order to take account of foliage on branch segments within the pathway the method of calculation needs to be modified from that presented here. Details are given by Gregoire *et al*. (1995).

Method BF3: Combined random branch sampling and importance sampling of large branches. In the above example we outlined how RBS can be used to estimate the biomass of tree foliage and branches

< 5 cm. By combining RBS and importance sampling an estimate of large branches can also be made thus giving an estimate of tree crown. This is achieved by estimating the biomass of branches along the pathway of the sub-sample and then using the unconditional inflation factor to estimate the total branch biomass. When this is combined with the foliage and small branch estimate a total crown estimate of biomass is obtained.

Branch volume is estimated using methods outlined in Section 2.4.1. Discs of branch wood are then sampled from each of the five pathways defined by random branch sampling. Location of the disc along the branch pathway is determined by importance sampling. Density is determined in each disc as described above and is applied to branch volume to obtain an estimate of branch biomass.

For each pathway, the following calculations are used (example of calculations given in Table 2.5):

1. Volume of each branch section is calculated using the diameter at the upper and lower nodes of the branch selected and internode length (Equation 3, Table 2.2). If diameter immediately below the second node it can be assumed that the sectional area at the node is equal to the sum of the sectional areas of branches emanating from the node see Equation 2.1). Thus, the equivalent diameter for the second node in Table 2.5 is $\sqrt{(33.5^2 + 9.0^2)} = 34.7$. Volume from the 1st node to 2nd node is then calculated as = 0.0722 m^3;

2. The inflation factor (or probability of selection) is calculated for the branch selected at the lower node in relation to the total cumulative value for branches at that node eg. Inflation Factor (1st node) = $(31^{2.5} + 5.5^{2.5}) / 31^{2.5} = 1.0133$;

3. The inflated volume is calculated for each branch section. eg. Inflated volume (1st node to 2nd node) = 0.0722 x 1.0133 = 0.0732 m^3;

4. Cumulative inflated volume is calculated for each section along the branch to obtain a total inflated volume for the branch pathway selected;

5. A random number between 0 and 1 is generated (eg. 0.6883) and multiplied by the total cumulative inflated volume (eg. 0.3111). Linear interpolation places this point 17 cm beyond the third node;

6. This location is marked on the branch, diameter is measured and a disc (approximately 10 cm thick) is cut. All pieces of wood and bark are collected and density is estimated according to the procedures outlined above;

7. An estimate of branch biomass is obtained by multiplying total inflated volume by the estimate of density; and

8. The procedure is repeated for each of the five pathways. The average value is used as the final estimate of large branch biomass while the standard deviation of the individual estimates can be used to estimate its error.

Note that in the above, branch volume is only estimated for the portion distal to the first node. This is appropriate for random branch sampling. Sometimes an alternative method is to make the initial choice about which branch is to be sampled, eg. when the crown needs to be stratified for some other purpose, or in the case of conifers where the main 'branch' (stem) is very much larger than other branches at the node. In such cases the volume from the butt of the branch to the first node needs to be included in the calculations. Thus diameter at the butt and length of the butt section needs to be measured. The conditional probability for this section is taken to be 1.0000.

Table 2.5 Example of calculations for randomized branch sampling and importance sampling of a crown

Node Number		DOB (cm)	$DOB^{2.5}$ (cm)	Cumulative $DOB^{2.5}$ (cm)	Branch selected	Conditional probability	Conditional inflation factor	Unconditional inflation factor	Length of branch (cm)	Volume of section (m^3)	Inflated volume (m^3)	Cumulative inflated volume (m^3)
1st node												
branch	1	31.0[A]	5350.6	5350.6[B]	✓	0.9869	1.0133	1.0133	85[E]	.0722	0.0732	0.0732
	2	5.5	70.9	5421.6								
random no.		0.9796[D]		5311.0[C]								
2nd node												
branch	1	33.5	6495.5	6495.5	✓	0.9639	1.0374	1.0512	80	.0697	0.0723	0.1455
	2	9.0	243.0	6738.5								
random no.		0.8845		5960.3								
3rd node												
branch	1	29.0	5428.9	5428.9	✓	0.8413	1.1886	1.2495	215	0.1114	0.1324	0.2779
	2	16.0	1024.0	6452.9								
random no.		0.1298		720.5								
4th node												
branch	1	17.5	1281.1	1281.1								
	2	7.5	154.0	1435.2	✓	0.0864	11.5805	14.4621	300	0.0145	0.1679	0.4458
	3	10.0	316.2	1751.4								
	4	4.0	32.0	1783.4								
random no.		0.7567		1349.5								
5th node												
branch	1	6.5	107.7	107.7								
	2	4.5	42.9	150.6	✓ cut	0.2746	3.6410	52.6659	220	0.0017	0.0062	0.4520
	3	2.0	5.6	156.2								
random no.		0.7319		114.3								
end of branch		0										
IS random no.		0.6883										0.3111

SECTION 3

ESTIMATING ROOT BIOMASS

3.1 INTRODUCTION

A wide variety of methods have been used to estimate root biomass but generally excavation and coring methods in conjunction with allometric relationships are used. This protocol does not describe all possible methods, but gives recommendations and examples for the selection of suitable (and practical) approaches for estimating total root biomass in support of the National Carbon Accounting System. For more details on these and alternative methods for root study the reader is referred to the classic work of Böhm (1979), the recent text on root methods edited by Smit *et al*. (2000) and the recent review by Snowdon *et al*. (2001).

Prior to initiating root studies in the field, background information on the environmental constraints to root development can be useful. Some of the soil structural features of importance are dry bulk density, pedality, soil strength, macroporosity, air and water porosity and micromorphology. Procedures for classifying soils are included in the Soil Sampling Protocol (McKenzie *et al*. 2001). Within a plant species, the root/shoot ratio, root decay and regrowth and distribution in the soil profile are dynamic and depend on environmental conditions such as soil structure, availability of nutrients and water, and may interact with the particular genetic character of the plant studied. Root biomass will normally have a high spatial (and temporal) variability, which is primarily associated with changes in stand development and soil properties. An understanding of this variability will also help in designing a root sampling methodology. For more information on sampling strategies and statistics associated with root sampling see Bengough *et al*. (2000).

3.1.1 OPERATIONAL DEFINITION OF ROOT BIOMASS

Root biomass, for the purposes of this protocol, is defined as those roots that are retained in a 2 mm sieve. Roots that pass through a 2 mm sieve are considered part of the soil carbon pool (McKenzie *et al*. 2001). The amount of roots that will be retained within a 2 mm sieve will be influenced to varying extents by the nature of sieving which is markedly affected by soil moisture content, i.e. wet versus dry. Consequently, it is recommended that the definition be extended to those roots retained within a 2 mm sieve after wet sieving.

Assessment of fine (< 2 mm) roots is of particular importance when estimating root turnover and carbon inputs in to soil via decomposition (especially for validation of models such as the Roth C model). However, fine roots generally do not make a major contribution to total root biomass in woody vegetation.

The definition of root biomass adopted here does not differentiate between live and dead roots owing to the inherent difficulties associated with distinguishing between them and the laborious nature of root-sorting.

3.2 METHODS

3.2.1 TWO-STAGE SAMPLING OF ROOTS

Usually roots of woody vegetation are sampled only to some root diameter or spatial limit. This is because: 1) it is impractical to retrieve all roots attached to any sample tree/shrub; and 2) generally the roots of neighbouring trees/shrubs will overlap. Consequently, for individual trees only roots greater than a certain diameter (eg. 25 mm) may be sampled, with smaller roots being ignored on individual trees. Alternatively, all roots within a defined soil volume are sampled, eg. all roots within a cylinder extending 1 m out from the stump centre or to the crown extent and to 1 m depth, may be sampled.

While such sampling is applicable to individual trees/shrubs, it is important that an estimate of other roots also be made. Consequently, a two stage approach would first sample roots attached to individual sample trees that have been destructively sampled for aboveground biomass, and then, a further sample of small and/or distal roots is taken.

Stage 1 - Sampling individuals

Excavation methods should be used for sampling individual trees and shrubs. For each species, at least 20 total root excavations, including lignotubers, root crowns and taproots are necessary for development of robust correlations with aboveground measures. Individuals selected for excavation should be representative of tree size aboveground and, for individual species, of differing site conditions.

The limit of root sampling on individuals should be carefully considered and based on practical considerations. If a root diameter limit is used then it should be set such that roots down to that limit can be efficiently retrieved. If a set volume of soil is used then the horizontal and vertical limits should be set so that most roots of sample trees are included. Sampling to the vertical projection of the crown may be relevant. This could be simplified by, for example, sampling to a radius equal to the average crown radius.

Root crown and major lateral roots of individual trees

Where feasible, a trencher or backhoe can be used to excavate the root system and to loosen it from the surrounding soil. A backhoe is essential for larger trees, particularly those with large lignotubers, and where sites have a large rock or clay component. The consistency of the soil will have a major influence on the success of the excavation method. Roots are much easier to expose in friable, well-structured or single-grain soils than in dense, hard soils. Where possible, moistening the soil overnight will greatly facilitate excavation. While excavations are laborious and time consuming, in very stony or dry sandy soils (where trenches may easily collapse) the excavation method is the only effective means to study root systems. Excavation of root systems will also generally require the use of shovels, fine brushes, hand-tools, and perhaps a water/air pressure system and hydraulic equipment. Careful removal of soil particles from the root system requires a wide variety of small hand tools, such as small metal forks, screwdrivers, dust brushes and brooms.

Once excavated, each root system can be divided into components of root crown, taproots and major lateral roots (Figure 3.1). Lateral roots are separated from the root crown beyond the basal swelling.

Corrections must be made for biomass lost during the excavation procedure. One approach is to count and measure diameters of all broken roots. The intact root systems are used to develop diameter to fresh weight estimates, which are then used as correction factors for root biomass lost from each broken end or to develop taper functions (see below). Alternatively, additional "scoops" or buckets (approximate volumes) can be sieved to collect broken roots, with a number of replicate samples (a minimum of five) to give a reasonable estimate.

In addition to the collection and weighing of roots, it is often helpful to record root forms *in situ* with a camera or using a sketch to note angles at which roots arise, particularly if future work requires further exploration of the fractal dimensions of roots (see below). During the excavation procedure, careful measurements and drawings should be made of the root system in both the vertical and horizontal planes. Such data can be invaluable for interpreting distributions and the spatial variability of coring data.

Other systems for sampling the roots of individual trees are outlined in Appendix 2. These rely on bulk excavation of the soil containing the roots of interest and subsequent separation of the roots from the soil. These methods are efficient means of estimating root biomass including, if desired, sampling in depth intervals and sorting of roots by diameter classes. However, they are not generally amenable to recording details of root architecture.

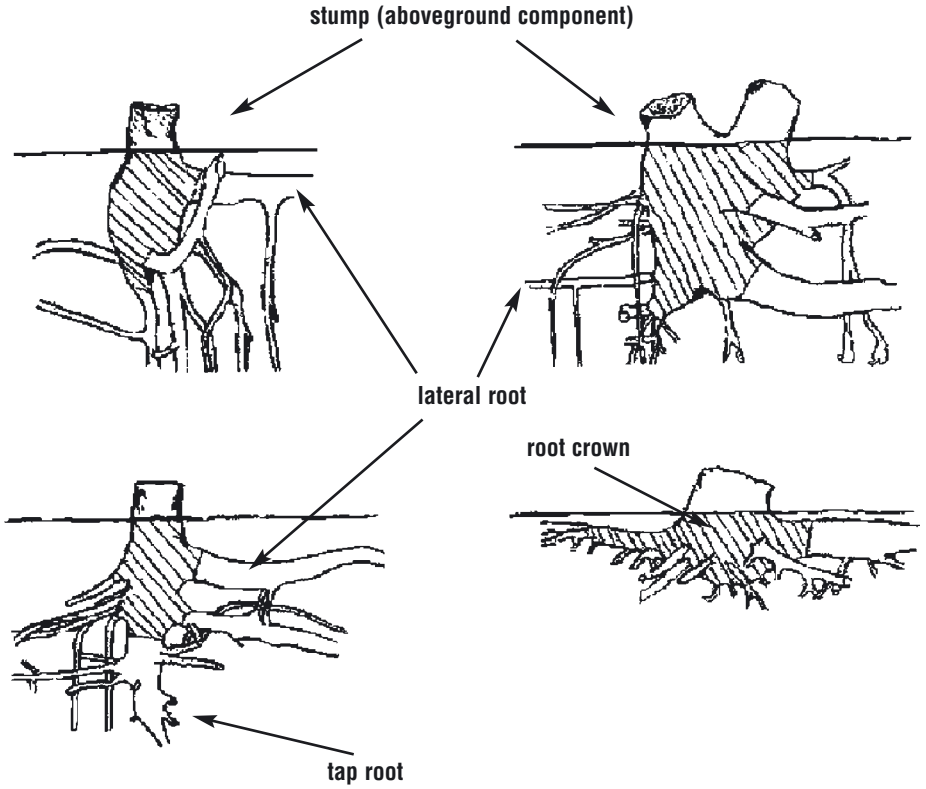


Figure 3.1 Examples of root stumps showing root crown (shaded) and other root components. Modified from Kimber (1974).

Stage 2 - Sampling small and/or distal roots

Roots not attached to individual trees/shrubs are best sampled by taking volumetric soil-root samples (coring, monolith and trenching methods). Because the source tree of sampled roots is not known this is best done in multi-tree plots. As a guide such plots should include at least 10 individuals from the overstorey. If individual trees were sampled to a set diameter limit then only those roots less than the diameter limit are retained. Thus if the diameter limit is, say 25 mm, the roots with a diameter > 25 mm are rejected from the samples. This is the method recommended by Newbold (1967).

Alternatively, if sampling of roots from individual trees is within a defined soil volume, eg. 1 m radius x 1 m depth cylinder, then sampling should include only roots outside the defined soil volume. This is the method of Burrows *et al*. 2000 (see also Appendix 2).

Figure 3.2 illustrates the case for trees that are individually sampled outside the multi-tree plots. For Example 1 (Appendix 2) each multi-tree plot included a cluster of 6-10 trees that were destructively sampled. Proximal roots of the individual trees were sampled by stump-pulling, other roots in the multi-tree plots being sampled by bulk soil sampling of the surface soil and soil coring of deep soil. One advantage of this approach to root sampling is that it is amenable to bulk excavation of all surface soil in the multi-tree plot. This may give a more accurate estimate of surface root biomass (highest concentration of roots occurs in the surface layers) than by sub-sampling. However, a disadvantage may be the extensive site disturbance involved. This is likely to be unacceptable in many ecosystems.

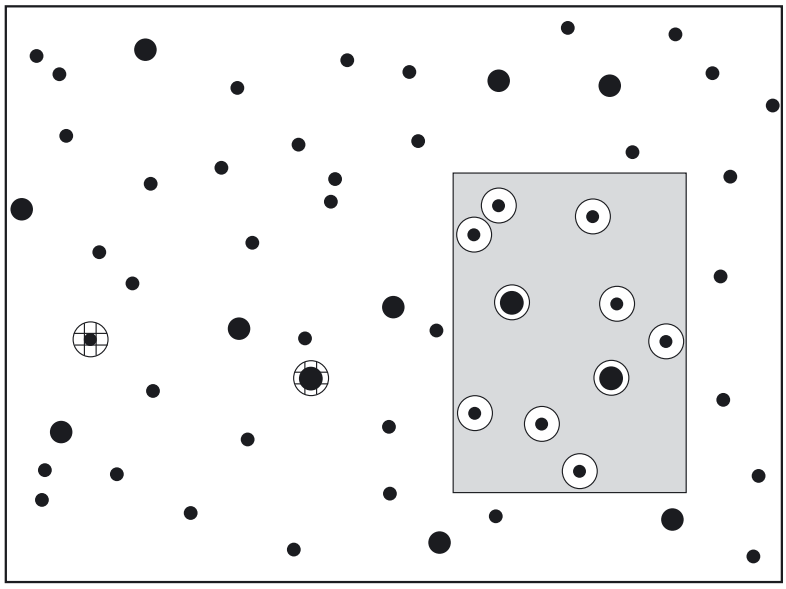


Figure 3.2 Example of root biomass sampling on individual trees and multi-tree plots based on spatial limit.

- Root sampling on individual trees (black dots) is confined to a circular area of fixed diameter around each sample tree (grey shade within grid areas). Sampling for other roots is confined to areas outside the circles but within the multi-tree plot (grey shaded area);
- Total root biomass in the multi-tree plot is estimated by summing the estimates for individual trees (using allometric relationships derived from the destructive sampling of individual trees) with that of the estimate for the grey-shaded area;
- Aboveground biomass in the multi-tree plot is also estimated from an allometric relationship developed from the trees that were destructively sampled; and
- Note that for adequate sampling of a whole population, more than the two destructive sample trees and the one multi-species plot indicated in this simplified example are necessary.

Note that roots of both over- and understorey vegetation[2] should be included when sampling of multi-tree plots. Aboveground biomass on these plots will also be estimated from biomass equations developed for overstorey species, and sampling of understorey species, whether treated collectively or individually. Thus, stand-level root:shoot ratios can be calculated.

3.2.2 USE OF VORONOI POLYGONS

Voronoi (Theissen) polygons are a popular method for modelling forest growth by apportioning potential growing space in a forest area among trees according to their size and position (Gadow and Hui, 1999). They may also be a logical means of defining the root sampling area of trees that have been harvested for measurements of aboveground biomass. However, the method does not appear to have been applied previously to estimation of root biomass.

A Voronoi diagram represents a continuous tessellation of an area into non-overlapping polygons. The polygons may be constructed from the perpendicular bisectors of lines joining a subject tree and its neighbours (Figure 3.3).

[2] The distinction between overstorey and understorey vegetation will normally be based on a height criterion. The understorey will often include small individuals of species that, when fully grown, form the overstorey. Selection of trees for destructive sampling should be restricted to trees large enough to be part of the overstorey.

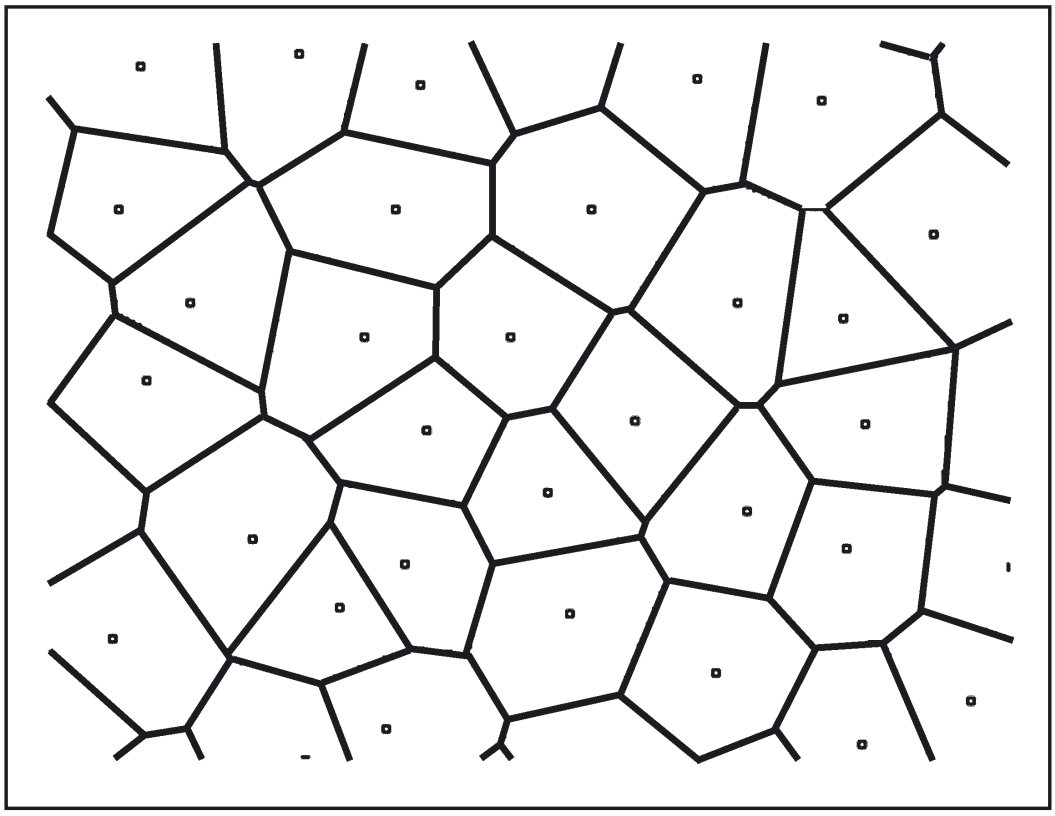


Figure 3.3 Example of a Voronoi diagram, illustrating boundaries between individual trees.

Weighted polygons may also be determined. For example, if root biomass increases in proportion to bole diameter, instead of using the mid-point between each individual tree and its neighbours, the following weighting function could be used:

$$BD_i = \left[\frac{DBH_i^2}{DBH_i^2 + DBH_j^2} \right] D_{ij} \qquad (3.1)$$

where BD_i is the distance to the perpendicular boundary line located on the straight line between the subject tree i and its neighbour tree j, and D_{ij} is the distance between trees i and j. This is a distance-dependent competition index originally proposed by Moore *et al*. (1973).

The example shown (Figure 3.4) was calculated with k=1 based on the assumption that the distance, BD, should vary in proportion to the DBH of the polygon tree, i.e. polygon area should vary with basal area of the polygon tree. The perpendicular boundary lines that form a polygon around a subject tree are located on the connecting (dashed) lines between the subject tree and its neighbours relative to the diameter of the trees.

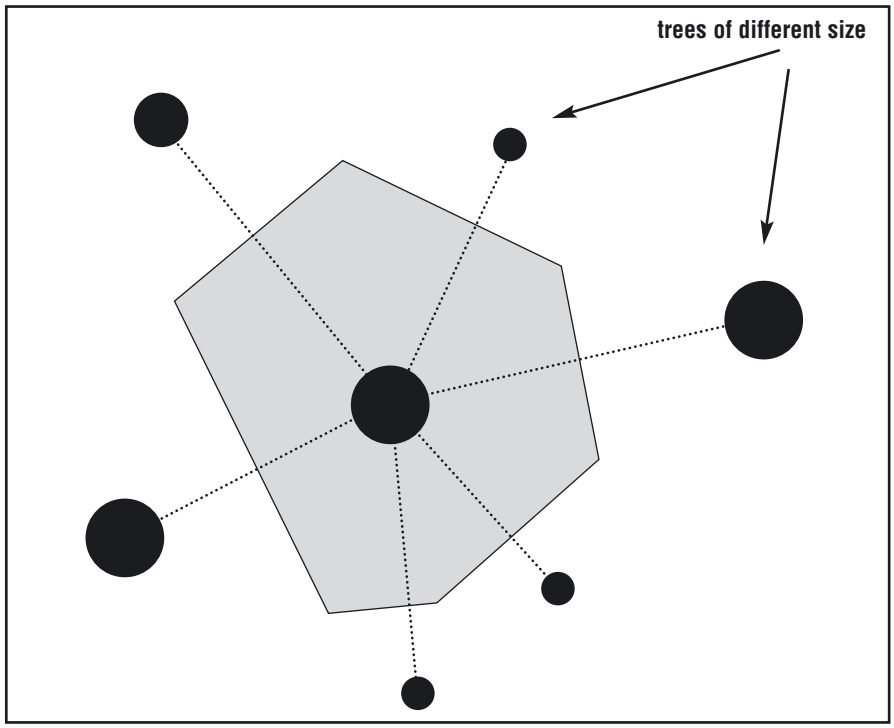


Figure 3.4 Example of a weighted area Voronoi polygon.

The entire polygon area for a destructive sample tree would need to be sampled. This could be achieved by excavating the entire area or alternatively, part of the polygon area could be excavated and part sub-sampled by coring.

Not all of the roots in a polygon area would originate from the polygon tree. Some roots will be from neighbouring trees and, conversely, some roots from the tree will have grown outside its polygon area. Therefore, it is important to note that a biomass prediction equation developed from Voronoi polygon sampling will predict root biomass in a tree's polygon area, and not necessarily the root biomass of the tree.

Understorey plants must be considered in addition to the overstorey. One approach is to define separate polygons for overstorey and understorey plants and therefore develop separate root biomass prediction equations for overstorey and understorey species. However, such an approach may not be practical, especially in diverse communities. An alternative is to define the polygons according to the size and position of overstorey trees only and include all roots (over- and understorey) when sampling an overstorey tree's polygon. In this way, all root biomass within an overstorey tree's polygon could be predicted from a single equation. If understorey roots are sampled in the polygons for overstorey trees then at least one ancillary predictor variable should be measured on the understorey, for example, the total area or percentage of projected foliage cover of the understorey within the polygon. In this case an equation would be developed to predict root biomass in an overstorey tree's polygon as a function of easily measured dimensions of both overstorey (eg. DBH, height) and understorey (eg. % cover).

Any equation developed from Voronoi polygon sampling will depend on the weighting function used to determine polygon boundaries. The equation, when applied in biomass inventory, would give an unbiased estimate of root biomass in the stand.

3.2.3 SUB-SAMPLING METHODS

Soil coring methods are often the simplest for taking volumetric soil-root samples and are generally collected using a hand auger or a solid metal or plastic corer (thin-walled tube) that is driven in to the ground. The diameter of the core is an important consideration, and it is recommended that cores be a minimum of 5 cm in diameter (≥ 10 cm preferred).

It should be noted that hand- auguring may not always give a sample that has a consistently accurate volume and there may be problems with roots on the edge of the hole not being cut cleanly and so biasing the sample. Therefore, before auger sampling is used routinely, estimates of root biomass obtained from auger samples should be compared with estimates obtained from other methods of soil sampling. Alternatively a thin-walled tube or a drilling rig (proline) should be used.

Other mechanised techniques include some types of augers driven into the soil by means of a heavy drop hammer and removed by a puller or jack screw. A pneumatic hammer or a portable drilling rig adapted to driving coring tubes may also be used. The relatively small diameters of the cores can be a disadvantage in sampling soil layers with low rooting densities, and there can be difficulty cutting through tree roots.

As a guide, in each multi-tree plot a minimum of ten randomly located cores should be sampled. Any roots in cores taken from multi-tree plots that would have been sampled on individual trees (based on root diameter or spatial limits) should be rejected. If a defined volume of soil were sampled on individual trees then, preferably, coring should sample roots in soil below the defined volume to the greatest depth of root growth. However, the depth achieved with reasonable effort will depend on soil conditions and equipment available.

An alternative to coring is excavation of set volumes of soil by machine (backhoe, excavator) and/or shovels. Thus a series of trenches can be dug across a site, or blocks of soil (monoliths) removed. Sampling to estimate root biomass per individual, either by repeated coring or by excavation of standard volumes, is particularly difficult on rocky sites. Another alternative is excavation of additional volumes of soil using a backhoe with a set number of sub-samples that are sieved to provide a general estimate.

3.2.4 SAMPLE PROCESSING

Sieving

Following hand-picking to remove larger roots, the remaining roots should be separated from soil by sieving. There are a number of different types of mechanised screen shaker units available to achieve this. Samples may be processed more quickly by preliminary sieving through larger mesh sizes (eg. 10 mm, 5 mm) to remove rocks, gravel and other non-root material.

In most cases, roots will require some washing in order to reduce soil contamination. The simplest and, in many cases, most economic method of separating roots from soil is a washing process with a jet spray of water aided by hand manipulation. In principle, the soil-root sample is suspended in water and poured over fine mesh screens (in this case, 2 mm) where the roots are retained and collected for further cleaning. It is generally not difficult to wash roots out of sandy soils, but the higher the clay content the more difficult the task becomes. There are numerous chemicals available to disperse soil colloids, predominantly pyrophosphates, which will accelerate the washing procedure (eg. Schuurman and Goedewaagen 1971, Oliveira *et al*. 2000). However, if roots are to be analysed for nutrients, particularly phosphorus and nitrogen, then many of the chemical dispersive agents will result in contamination and should be avoided. Soaking soil samples overnight can also increase the ease of separating roots from clayey soils.

In addition to separating roots from mineral soil, there is often a large component of organic matter and charcoal in both forest and woodland soils that will require removal by hand. Organic matter can often be removed from soil samples (particularly when separating fine roots) by flotation and scooping off the floating layer and by successive "panning" in a tray of water to float off organic matter. In some cases the organic debris and roots are of similar density and cannot be separated by flotation. Additionally, the organic debris may adhere to the roots, often attached by fungal hyphae or root mucilage/exudates. In these cases roots must be separated by hand picking.

Root size classes

Roots are generally divided into coarse, medium and fine categories, the proportions of which will influence both estimates of total standing root biomass at any point in time, and the rate at which those same roots senesce and are incorporated into the soil carbon fraction (turnover). The relative root size distribution also changes with depth. It is recommended that where appropriate, roots be separated into root crown, tap root and major laterals and also be divided in to a number of root diameter classes (eg. < 2, 2-5, 5-50, > 50 mm.)

The allocation of biomass by root class will assist in improving models of root decay and input of carbon into soil. The separation of the smaller diameter classes is particularly important to reconcile some of the overlap that occurs when estimating carbon estimates in soils (McKenzie *et al*. 2001). Separation of samples in to size classes is most rapidly achieved using a combination of hand sorting and wet sieving.

3.2.5 ROOT DRY WEIGHT

To determine dry weight, the washed and cleaned roots, or a representative sub-sample thereof, are dried in an oven at 70°C until no further weight change occurs (or until less than 1% weight change after 24 hours). For many larger roots this may require drying for days to weeks. Even after thorough washing and cleaning soil particles may still adhere to roots. After initial drying, larger roots should be brushed off with a paintbrush before final weighing. There will be some degree of soil contamination on all roots, particularly on smaller roots in very clayey soils. Therefore, a sub-sample of dried roots can be ashed in a muffle furnace at high temperature to combust organic matter. The weight of ash residues is then deducted from the subsample dry weight to give the correct weight of the roots. This correction factor should be determined for each set of site conditions, and for individual operators involved in root washing and sieving. All roots should be sub-sampled for determining dry and fresh weights.

3.2.6 ROOT CARBON CONTENT

Several estimates of the carbon content of woody roots are already available (Gifford 2000). If an independent measure is required, sub-samples of oven-dried roots should be ground to a fine powder (eg. using a vibratory ball mill). Sub-samples of the ground roots should then be dried again at 70°C for at least 24 hours and then samples analysed for carbon content (%) using a combustion method (eg. Automated Carbon Analyser-Mass Spectrometer or similar methods).

3.3 SUMMARY

1. Roots are defined as all live and dead belowground biomass > 2 mm in diameter (retained in a 2 mm sieve);
2. Stratified sampling should be used to select sites covering the range of site conditions and, within sites, the range of age and tree sizes. Site conditions should be described, particularly disturbance history and soil conditions;
3. Two-stage sampling can be used to estimate root:shoot ratios at the stand level:
   (a) Roots of individual sample trees are sampled to a consistent root diameter or soil spatial limit.

(b) Roots not sampled on individual sample trees (destructive sampling for above- and belowground biomass) should be sampled in multi-tree plots. This should include roots of understorey and overstorey vegetation.

(c) Aboveground biomass is also estimated on the multi-tree plots from biomass prediction equations.

(d) Root:shoot ratios for multi-tree plots is calculated;

4. An alternative to two-stage sampling is the Voronoi polygon approach to develop individual tree prediction equations for root biomass:

   (a) Weighted area Voronoi polygons area is defined for each destructive sample tree and then thoroughly sampled for root biomass.

   (b) Biomass prediction equations developed from Voronoi polygon sampling will predict root biomass in a tree's polygon area, not root biomass of the tree;

5. Excavation is used to sample the roots of individual trees, either sampling all roots in a defined soil volume (two-stage sampling and Voronoi sampling) or to sample roots to a defined diameter limit;

6. Soil sub-sampling methods are used to sample roots not sampled by excavation, i.e. smaller and/or distal roots. Set volumes of soil are removed in cores, monoliths or trenches; and

7. Sieving is used to separate roots from soil using a variety of techniques. Excavated soil may be first sieved through a coarse sieve.

In some systems fine root dynamics can have a significant effect on total root biomass. Where rainfall is very seasonally variable, measurements may be needed to estimate annual minima and maxima for fine root biomass.

SECTION 4

REPORTING REQUIREMENTS

4.1 METHODS

An outline of the scope and objectives of each particular study should be reported. A detailed description of the methods used to estimate biomass of the various components studied should be given. Where applicable, reference should be made to methods outlined in this Protocol and the specific way they have been applied in the study. Details should be given of any modifications or alternative method that has been used. Methods used to select sample plots and trees should be given. Where applicable, definitions should be given of the boundaries used to define different biomass components, eg. diameter limits for branch and root components.

4.2 PLOT INFORMATION

4.2.1 PLOT ESTABLISHMENT AND LOCATION

The preferred option for locating plots in the field is by use of a Global Positioning System (GPS). This enables efficient and accurate placement and relocation of permanent sample plots. Differential correction should be used particularly in natural forest and thick vegetation. Alternatively, plots can be located by compass bearings and distances from known landmarks.

Plot sizes are defined in the horizontal plane. Thus, measurement tapes need to be held horizontal when establishing plots on sloping ground. If this is precluded on a steep slope, correction tables can be used to derive plot dimensions suitable for measurement on the slope (eg. Ellis and Hayes 1997, Appendix 4).

Plot centres or corners should be marked so that plots can be easily relocated. In some cases unobtrusive markers, such as a buried piece of pipe, will be needed so that the plot will not receive any special treatment during forestry operations.

4.2.2 PLOT DESCRIPTION

Description of the stand(s) used for carbon estimation should include as a minimum:

- General location of sample stands;
- Forest or vegetation type (eg. Table 14a; McDonald *et al*. 1990);
- Land use classification (Baxter and Russell 1994) Refer [http://www.brs.gov.au/land & water/landuse/class.html]; and
- Landform pattern and element (eg. Tables 4, 5; McDonald *et al*. 1990).

Additional detail should be recorded for individual plots including:

- Plot location including latitude, longitude and map reference;
- Instructions for precise re-location of sample plots;
- Plot size, shape and orientation;
- Slope, aspect, elevation and topographic position;
- Forest or vegetation type including major species;
- Stand age if even aged or growth stage;
- Disturbance history especially time since last disturbance;
- Condition of understorey and forest floor;
- Stand density as basal area and stocking;
- A list of diameter and other measurements for trees within plots; and
- A measure of stand height.

If a detailed sampling for soil carbon (McKenzie *et al*. 2001) is not being made a technical classification of soil conditions (eg. Turvey 1987) is required. This should include:

- Parent rock type;
- Soil texture profile;
- Depth to impeding layer; and
- Other readily available soil information.

4.3 BIOMASS COMPONENTS

4.3.1 SAMPLE TREE DESCRIPTION

A description of each sample tree should be given including:

- Species;
- Status in the community (eg. dominant, suppressed, juvenile);
- General health;
- Presence of defects, hollows, etc.; and
- Crown development and status.

Stem and other measurements potentially useful for the development of allometric or linear equations for predicting biomass should be given. Measures other than those necessary for the current study should be considered particularly if they would enhance the capacity of the data to be amalgamated with that derived from other studies. Tree measurements could include:

- Diameter at breast height (DBH), which is the most commonly used variable;
- Diameter at other locations, eg. 0.1 m, 0.3 m, base of green crown;
- Height;
- Length of merchantable bole;
- Volume; and
- Crown width, depth, area, or volume.

Whatever the measurement may be, it is important that standard forestry protocols for making those measurements should be used (eg. Anon. 1995, Ellis and Hayes 1997, Wood *et al*. 1999).

4.3.2 ABOVEGROUND TREE BIOMASS COMPONENTS

For each tree a table containing the estimates of biomass in each component should be given. This should include the biomass of sub-components when these have been estimated separately, eg. stemwood and bark, merchantable and non-merchantable bole; different foliage age classes; etc. Where possible an estimate of error in the estimate of each biomass component should be given (eg. see Section 2).

4.3.3 ABOVEGROUND UNDERSTORY COMPONENTS

When understorey species have been sampled individually for the development of regression estimators then details should be given of measurements for various independent variables (eg. height, diameter) and biomass components analogous to those required for overstorey trees. Details of any regression equations developed from these data should be given particularly if they have been used to derive plot-based estimates for the study. Where possible, plot-based estimates of understorey biomass, whether derived from regression estimates or some other method (eg. Catchpole and Wheeler 1992), should be accompanied by an estimate of errors associated with the estimate.

4.3.4 ROOT COMPONENTS

Information on root biomass will usually consist of:

- estimates of biomass of roots known to be attached to a particular tree or shrub; and/or
- estimates of biomass of roots of unknown attachment.

In both cases the roots might be assigned to different compartments on the basis of diameter or some other criterion, eg. health, species. In the first

case a clear association should be made between the biomass measurements and details of the tree to which they are attached (see Section 4.3.1). In the second case an estimate of error should be provided. This can be calculated from data for individual sub-samples such as soil cores.

4.3.5 REGRESSION RELATIONSHIPS

Details of any regression relationships developed during the study should be reported. These could include regressions developed to aid estimation of individual components within trees eg. estimation of branch weight from branch diameter, or those developed for the estimation of biomass in non-sample trees. The report should include:

- Description of the equation and the method used for estimating parameter values;
- Estimates of the coefficients and their standard errors; and
- Error mean square for the regression and either the total or regression mean (or total) squares.

4.4 STAND ESTIMATES

The results for stand based estimates of biomass should be reported according to the general principles developed above for individual trees. Attention should be given to providing stand based estimates of potential independent variables such as stand basal area, stocking, height etc., in addition to information about biomass in different components. Estimates of errors should be given wherever possible.

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APPENDIX 1

ESTIMATION OF UNDERSTOREY AND VEGETATION WITH IRREGULAR STRUCTURES

A1.1 INTRODUCTION

In arid and semi-arid Australia, predictions of either individual tree or shrub or stand mass based on diameter are likely to be unreliable. The two prime factors that mitigate against standard approaches are:

1. tree and shrub form and,
2. the effects of past climatic and disturbance events and biological processes on form.

The first factor is obvious – trees and shrubs in woodlands and shrublands are seldom regular in shape or form. Instead, form varies widely, even wildly, and without substantial modification, use of standard approaches to estimate stem volume (eg. Newton's formula, Smalian's formula, taper functions, general allometric models) will be of marginal value. The modifications required are essentially that each section of stem or branch be treated independently and/or that different independent variable(s) may be needed to predict mass. In many instances, sections of stem or branch will be less than one metre in length and, clearly, a larger amount of work is required to estimate volume and thence mass of irregular single trees/shrubs than the trees of regular form found in more mesic climates.

The second factor means that forestry approaches will fail without substantial modification because underlying physiological/anatomical principles govern the magnitude of diameter-mass relationships. Hence, while the cross-sectional area of sapwood and leaf mass and area are probably related, there is poor empirical evidence that this is the case for most of the dominant tree and shrub species in arid and semi-arid Australia. More importantly, stem or branch diameter is frequently (as often as 75% of cases) poorly related to stem or branch mass owing to the progressive decay of heartwood. An equally important cause of failure of a simplistic allometric approach is the propensity for branch abscission in response to a wide range of influences such as fire, drought and insect attack. The great majority of mature eucalypts (and many Acacias) in arid and semi-arid Australia consist of stems and main branches that once supported other secondary branches which have long since been shed. Diameter fails to reliably predict current mass owing to the past formation of wood ('pipe') that is no longer required to support the current foliage. This difficulty can only be overcome by the approach described above – measuring the volume/mass of individual segments. Taper functions are of limited use because there is often a 'step' in diameter at each branch node, especially if the node is the site of an abscised branch. Allometric models based on diameter can be used to predict branchwood and leaf mass, but only for branches where the underlying principles still hold. In practise, this is likely to be smaller, younger branches on trees and younger shrubs.

Two eucalypts, River Red Gum (*Eucalyptus camaldulensis*) and Coolabah (*E. victrix*) are widespread throughout arid and semi-arid Australia and dominate creek- and stream-lines where they may comprise the bulk of ecosystem biomass. These species seldom grow with regular form and may live to considerable age, surviving fires, floods and drought but at the cost of increasingly irregular form. Similarly, a great many species of eucalypts – including species that may be grouped as mallee's, gimlet's and bloodwoods – dominate vast areas, suffer the consequences of disturbance or form, and are long-lived. The same applies to the large numbers of Acacia sp. that progressively replace eucalypts as the dominant woody vegetation as conditions become more arid. For all these species, as well as the other woody shrubs (eg. *Eremophila* sp.) that may comprise the bulk of above-ground biomass, the notes provided in this section will be a useful starting point to the assessment of biomass.

By necessity, this section is less *prescriptive* than other sections of this protocol and more *descriptive*. Many of the techniques for developing robust regression models can be applied to trees in the arid and semi-arid regions, provided they are applied at the correct scale and provided due recognition is given to the underlying principals that allow diameter (or any other independent variable) to be a useful predictor of wood and foliage mass.

This Appendix is written to provide guidance to a set of methods that allow both standard forestry approaches to be employed and, alternative approaches based on interpretation of crown area.

A1.2 SITE AND SAMPLE SELECTION

As described elsewhere statistically rigorous development or calibration of predictive models requires a weighted selection of trees/shrubs for destructive sampling, based on the full range of mass and size of individual plants and on the proportional distribution of trees/shrubs within diameter/size classes. In standard forestry techniques, diameter, and sometimes height, are normally used as a surrogate for all other dimensions for the purposes of stratification.

Experience suggests that for the irregular trees and shrubs in arid and semi-arid regions, measurement of *c*rown size in addition to diameter and height will help explain biomass distribution. This requires an estimate of crown volume (*l*ength x *w*idth x *d*epth) or, if resources are extremely limited, area (*l* x *w*). As with standard approaches, the researcher needs to establish the proportional distribution of trees or shrubs in each diameter or size class and then select sample trees or shrubs according to the principles laid down in Appendix 3.

Sites should encompass the known range of productivity of each species (as a function of soil type and rainfall), and variations in species form (eg. mulga may be classed into at least 4 forms).

A1.2.1 REQUIRED MEASURES

1. Disturbance effects (fire, drought, mining, grazing) should be recorded if known;
2. Either multiple transects and 'plot-less' methods or replicated large plots (size determined by standard techniques) can be used to estimate tree and shrub density and the proportional distribution of each species within diameter/size classes;
3. For all trees, measure height, basal diameter (0.1 m), DBH (1.3 m), diameter at crown break, crown depth and crown dimensions);
4. For all shrubs, measure basal diameter, height, crown dimensions; and
5. Select sample trees and shrubs, for detailed measurements as described below.

A1.3 ESTIMATING BIOMASS OF INDIVIDUAL WOODY SHRUBS

A variety of methods are available for estimating biomass of shrubs and understory components in woody ecosystems (Catchpole and Wheeler 1992). Methods include:

- Destructive sampling of quadrats;
- Line and planar intersect methods;
- Calibrated visual estimation techniques; and
- Double sampling using regression estimators.

Guidelines for only the last are given here. Experience suggests that the most useful methods are variants of those described elsewhere in this Protocol.

For most shrubs, a 'cut and weigh' approach (see also Complete Harvest Method, Section 2.3) works reasonably well for shrubs up to a height of about 4 m that can be handled, with some difficulty, by a team of 3-4 persons. In short, sample individuals of

each species should be measured for diameter at base (or a standard height above ground eg. 10 cm) and crown dimensions and height, and then cut at ground level. Total fresh weight should be measured in the field, as quickly as possible, and then a representative sub-sample (i.e. retaining the overall proportions of wood:foliage) can be selected, weighed and taken away for later separation into components. If possible, the foliage should be assessed for total leaf area as well as dry mass (oven dry weight) whereas the dry mass of wood is all that is required.

The methods outlined below for trees naturally become appropriate once 'shrubs' become too big to be 'cut and weighed'. The generally multi-stemmed nature and convoluted pattern of branching of shrubs may well suggest that a path selection and importance sampling approach, as described in Section 2.4.2 would be the easiest approach for large individuals.

Fortunately, the total and component masses of a great many irregularly shaped shrub species does not depart greatly from that which can be predicted on the basis of simple measurements of diameter or cover (see also Brown 1997). Usefully, quite a range of different species from a single genus or related genera may fit a single predictive model. In light of the above discussion, this generalization holds best for relatively short-lived shrub species that have not been substantially affected by fire, drought etc.

A1.3.1 REQUIRED MEASURES

1. Record height, crown dimensions and basal diameter of each shrub (if more than one stem, measure all stems ≥ 1 cm);
2. Harvest entire shrub and separate into wood and leaf components (at least 10 of each species at each site);
3. Weigh total components and then sub-sample for determination of conversion factor for estimation of wood and leaf biomass; and
4. Sub-sample leaf biomass to estimate leaf area and LAI.

A1.4. ESTIMATING ABOVE-GROUND BIOMASS OF INDIVIDUAL TREES

The major variation from methods described in Section 2 is the requirement for a greater intensity of measurement of dimensions of stem and branch segments.

By way of examples, in recent assessment of *E. camaldulensis, E. victrix* and *E. leucophloia* stands and mulga woodlands in the Pilbara, W.A., the stem of every eucalypt sampled approximated a tapered cone for a few metres above ground level, at most. Beyond this height, stems were either non-existent or multiple or greatly deformed. Each tree contained either a main stem or one or more main branches that were either substantially hollow or at least partially decayed. The main branches of each tree (that together comprised a greater proportion of total mass than the stem) were highly convoluted in form and each tree had previously lost large secondary (or even primary) branches. Experience suggests that hollow or rotting stems and branches present an almost impossible problem to overcome. Taking discs or other samples for wood density measurements, is often highly destructive and always time-consuming, visual inspection can seldom reveal the state of internal decay. We suggest that apart from the 'chance' (i.e. that the selected disc or discs truly represent all the wood) incorporation of the true density measurements, rot will remain a source of error.

It is quite clear, from studies in both Australia and overseas that for each species and for each form of each species, a separate analysis is required.

The more straightforward issue of irregular form has been dealt with previously (eg. Valentine *et al*. 1984) and we suggest de Gier (1989); de Gier and Sakouhi (1996) provide excellent descriptions of path selection, and an importance sampling approach (see also Section 2.4). The method does

not provide estimates of leaf mass or area and so will be unsuited to some purposes although a combination of this and the methods outlined below may allow reasonable estimates of leaf mass and area to be obtained using regression analysis. Critically for remote locations, the path selection approach is very efficient in time usage: average time per tree is only about thirty four minutes for a crew of two (De Gier and Sakouhi 1996), only increasing slightly with increasing DBH, resulting in on-the-spot estimates of volume and fresh weight. Furthermore, the approach needs little more than a power saw, a balance and a portable computer for making calculations in the field.

A brief description of a more time-consuming and labour-intensive approach that yields more accurate estimates of wood volume and leaf mass and area is described below. This accounts for each 'step' in diameter, and thus volume, of each branch. This approach also allows for the development of regressions to predict leaf and wood mass and leaf area for branches where such relationships still hold.

A1.4.1 REQUIRED MEASURES

Single-stemmed trees – regular form

1. Record – height, crown dimensions, basal diameter at 0.1 m, 1.3 m (DBH), diameter at crown break (or stem split), height at crown break/stem split;
2. Measure basal diameters of every order of branching (eg. division in to 2 stems, then branches on each main stem) – record distance from branch/stem base that estimation was made – and measure diameter at least 3 times along each branch (to calculate individual segment volumes);
3. It is critically important to measure diameter at each 'branching node', irrespective of the presence of dead or live sub-branches. Diameter should be measured immediately below and above each 'node' especially where one of the sub-branches is dead or has been abscised;
4. For selected branches (at least 3, representing basal diameter size range), separate leaves from wood to calculate leaf biomass and wood biomass in each;
5. Sub-sample fresh weight of stem and branch wood (discs up stem, recording distance from base where taken) to estimate dry weight and to calculate wood densities; and
6. Sub-sample leaves to estimate dry weight and to calculate total leaf area (and LAI)

Multiple-stems – regular form

1. Record – height, crown dimensions, basal diameter at 0.1 m, 1.3 m (DBH), diameter at crown break (or stem split), height at crown break/stem split of every individual stem;
2. Where stems divide at > 0.1 m from base, record total basal diameter at 0.1 m and then at stem division (as above for single stem species);
3. Record diameters for each individual stem at 0.5 m intervals until a branch is reached, also recording DBH (1.3 m) where possible;
4. Measure basal diameters of every order of branching (eg. division in to 2 stems, then branches on each main stem) – record distance from branch/stem base that estimation was made – and measure diameter at least 3 times along each branch (to calculate individual segment volumes);
5. It is critically important to measure diameter at each 'branching node', irrespective of the presence of dead or live sub-branches. Diameter should be measured immediately below and above each 'node' especially where one of the sub-branches is dead or has been abscised;
6. For selected branches (at least 3, representing basal diameter size range), separate leaves from wood to calculate leaf biomass and wood biomass in each;

7. Sub-sample fresh weight of stem and branch wood (discs up stem, recording distance from base where taken) to estimate dry weight and to calculate wood densities; and

8. Sub-sample leaves to estimate dry weight and to calculate total leaf area (and LAI).

Irregular form

1. Record – height, crown dimensions, basal diameter at 0.1 m, 1.3 m (DBH), diameter at crown break (or stem split), height at crown break/stem split of every individual stem, if tree is leaning over and angle to ground surface;

2. Where stems divide at > 0.1 m from base, record total basal diameter at 0.1 m and then at stem division (as above for single stem species);

3. Record diameters for each individual stem at 0.5 m intervals until the first main branch is reached, also recording DBH (1.3 m) where possible;

4. Measure basal diameters of every order of branching (eg. division in to 2 stems, then branches on each main stem) – record distance from branch/stem base that estimation was made – and measure diameter at least 3 times along each branch (to calculate individual segment volumes);

5. It is critically important to measure diameter at each 'branching node', irrespective of the presence of dead or live sub-branches. Diameter should be measured immediately below and above each 'node' especially where one of the sub-branches is dead or has been abscised;

6. Record diameters of dead limbs or where noticeable decay or hollowing of limbs;

7. For selected branches (at least 3, representing basal diameter size range), separate leaves from wood to calculate leaf biomass and wood biomass in each;

8. Sub-sample fresh weight of stem and branch wood (discs up stem, recording distance from base where taken) to estimate dry weight and to calculate wood densities; and

9. Sub-sample leaves to estimate dry weight and to calculate total leaf area (and LAI).

A1.5 EXAMPLES

A1.5.1 SNAPPY GUM (EUCALYPTUS LEUCOPHLOIA)

1. Draw sketch of tree, illustrating branching pattern (see Figure 4.3); and

2. Record tree height, crown dimensions (including depth), height to crown break (if applicable).

Number the branches and record, for each branch, the length with wood, i.e. until where the branch divides/ branches again

3. For the wood component of each branch (i.e. the section for which length was measured in 2. above), measure the diameter at 0.5, 1.0, 1.3 m and so on, avoiding measurements at bulges around sub-branches. Remember to measures diameter immediately below and above each node; and

4. For each main branch (at 2. above), count the number of sub-branches and record their basal diameters (again, avoiding measurements around "bulging").

Destructively sample 2-3 sub-branches for each tree. For each of these sub-branches, measure foliage (mass and area) and total wood (mass only).

A1.5.2 MULGA (ACACIA ANEURA)

1. Note form of tree (1-4, where 1= single-stemmed, 2=multiple-stemmed, 3=pole, and 4=multi-stemmed shrub < 2 m in height);
2. Draw sketch of tree, illustrating branching pattern;
3. Record tree height, crown dimensions (including depth), height to crown break (if applicable);
4. Number the branches and record, for each branch, the length with wood i.e. until where the branch divides/branches again;
5. For the wood component of each branch/stem (i.e. the section for which length was measured in 2.above), measure the diameter basal diameter (if possible) and at 0.3, 0.5, 1.0, 1.3 m and so on, avoiding measurements at bulges around sub-branches;
6. For each main branch (at 2. above), count the number of sub-branches and record their basal diameters (again, avoiding measurements around "bulging");
7. Destructively sample 2-3 sub-branches for each tree. For each of these sub-branches, measure foliage and total wood for each. The basal diameters of the sub-sampled branches should incorporate the range from the tree; and
8. Ignore dead branches other than ensuring measurements of diameter immediately below and above their point of insertion (see above).

APPENDIX 2

SOME PRACTICAL EXAMPLES OF ESTIMATING ROOT BIOMASS

A2.1. PLANTATION PINUS PINASTER AND EUCALYPTUS GLOBULUS IN WA

(Contact: Peter Ritson, Department of Conservation & Land Management, WA)

A2.1.1 SOILS

Soil types sampled include deep sands, duplex sand over clay, and gradational soils. Often there are soil layers with a high gravel content (> 50% ferruginous nodules). Some soils have hardpans (ferricrete or silcrete).

A2.1.2 SAMPLE TREE SELECTION

Sample trees are grouped in plots of 6-10 trees (cluster sampling). Each plot is selected to be representative of the general stand condition and contain trees from the range of crown dominance classes (suppressed to dominant). A variety of plots (17 *P. pinaster* and 12 *E. globulus*) have been sampled to cover the range of tree age, site quality and planting arrangements for the two species.

A2.1.3 ROOT SAMPLING

Each sample tree is allocated an area (sub-plot), the boundaries being mid-way between the sample tree and adjoining trees. Root extraction is then a three-step process (Fig. 4.6):

Step 1: The surface soil from around the stump and out to the sub-plot boundaries is excavated (back-hoe or small excavator). This soil is put through a coarse sieve comprising wire netting (25 mm or 50 mm holes) suspended on 100 mm grid weldmesh (2 m x 1 m field table). Excavation depth is sufficient to include all the zone of surface root proliferation and is typically around 0.6 - 0.8 m. Most of the roots can be picked off the sieve table for weighing but some, generally smaller roots, drop through the coarse sieve. If roots are difficult to separate from the soil, eg. some finer textured soils, then more effort is required for sieving and more roots pass through the sieve;

Step 2: Once the lateral roots from around the stump are cut, the stump and attached tap roots are pulled out (chain to back-hoe bucket) for weighing. Some of the roots break off towards their small ends. Any soil on the stump and attached roots is removed by washing or brushing prior to weighing. Generally the same machine that is used to pull the stump out is used to weigh the stump (spring balance between the bucket and the stump); and

Step 3: The excavation pit is back-filled with the excavated soil and soil cores taken to assess roots missed in Steps 1 and 2. Where possible, a post-hole digger (150 mm or 100 mm diameter bucket) with motor-power head is used. In very hard soils a trailer-mounted drilling rig is used (hollow auger system, 45 mm diameter core). All core samples are put through a fine (2 mm) sieve to extract roots for weighing. Thus the coring procedure samples roots in the back-fill (generally small roots that passed through the coarse sieve) and deep roots. Coring proceeds to a depth where no roots are apparent in a 1 m length of soil sample, or a layer too hard to drill is reached. Maximum coring depth has been 6 m.

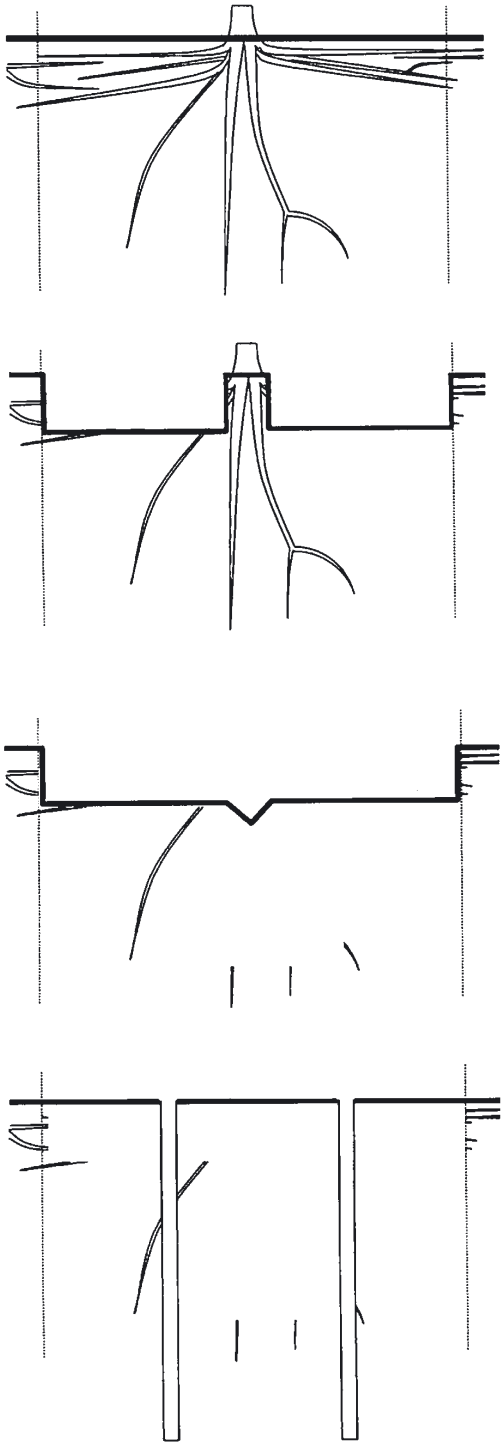

Diagram of stump and large roots with land surface (▬) and plot boundaries (.....) indicated. Medium and fine roots not drawn.

Step 1. Surface soil excavated and put through coarse (25 or 50 mm) sieve to extract most roots. Some (mostly small) roots pass through the sieve.

Step 2. Stump and attached taproots pulled out. Some roots break off towards small end.

Step 3. Pit back-filled with excavation soil and soil cores taken to sample roots missed in Steps 1 & 2. Soil cores put through fine (2 mm) sieve.

Figure A2.1 Root sampling procedure

A2.2 EVERGREEN EUCALYPTUS SAVANNA

(Contact: Derek Eamus, University of Technology, Sydney)

Belowground biomass of an evergreen *Eucalyptus savanna* was estimated at a site close to Darwin, NT using the trench method. A total of 8 trenches were dug with 2 trees of either *E. tetrodonta* or *Erythrophleum chlorostachys* located in each trench. One trench contained 3 trees; the total number of trees excavated was therefore 17. Their diameter at breast height (DBH) ranged from 4.1 cm to 20.8 cm.

Briefly, the method used was as follows:

Trenching

A pair of trees of either *E. tetrodonta* or *Erythrophleum chlorostachys* were identified that were 3-6 m apart. A rectangle was painted on the ground such that each side of the rectangle was 1 m away from each tree (Figure A2.2). The DBH of each tree was recorded and then the tree was cut down.

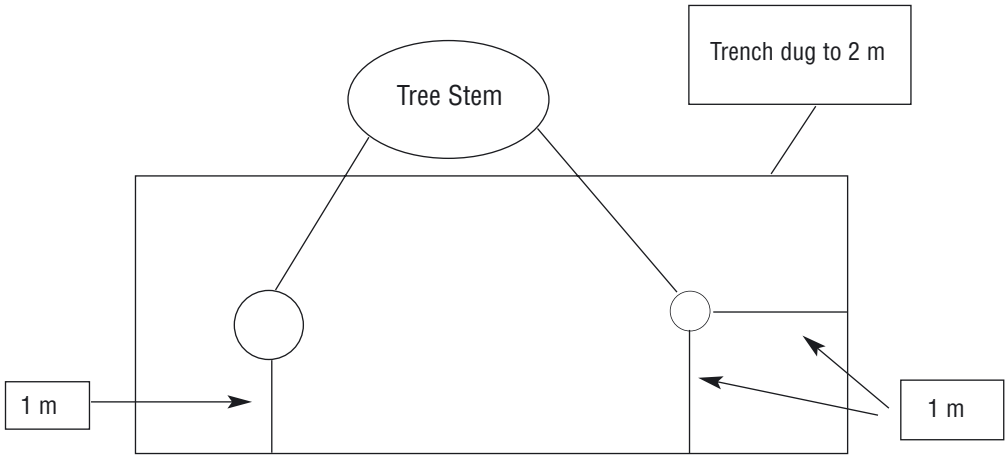

Figure A2.2 Diagram of the trench excavated around each pair of trees in the Darwin study.

Each trench was between 5 and 55 m^3 in volume, depending on the size of the trees being excavated and the distance between each tree of the pair. Each trench was excavated with a mechanical digger. The excavator bucket measured 600 mm wide and its volume was approximately 0.2 m^3. Individual "scoops" of soil were removed sequentially and deposited in individual piles on cleared areas either side of the trench. The origin of the "scoop" was recorded and the soil thus identified. Individual scoops were kept separate. Each trench was excavated to a depth of 2 m.

A2.2.1 COLLECTION OF ROOTS AND ABOVEGROUND PARAMETERS

A randomly collected sub-sample (typically 40% of the original volume) of each scoop of soil was spread thinly on a large sheet of plastic. Four assistants then extracted all roots visible in this thin (< 0.5 cm) layer of soil by manually trawling through the soil, for exactly 10 minutes. Previous experience had shown that doubling this time only increased the mass of roots obtained by a small fraction. Roots were classified into fine (< 2mm) and coarse (> 2mm) roots, dried in an oven and their dry weight recorded. Most eucalypts in northern Australia possess a large lignotuber and very large (diameter > 4 cm) tap roots that extend vertically. The mass of these roots and lignotuber was measured separately for each tree.

Table A2.1 Characteristic features of excavated trenches

Trench No	Species	No of trees	Area (m^2) excavated	Volume (m^3) excavated
1	*Erythrophleum chlorostachys*	2	27	54
2	*Erythrophleum chlorostachys*	2	22.54	45
3	*Erythrophleum chlorostachys*	2	16	9.3
4	*Erythrophleum chlorostachys*	2	8.8	5.3
5	*Eucalyptus tetrodonta*	2	13.5	27
6	*Eucalyptus tetrodonta*	2	13	26
7	*Eucalyptus tetrodonta*	3	8.4	5
8	*Eucalyptus tetrodonta*	2	8.3	5.6
Total			**117.5**	**177.2**

A2.3 OPEN TREE STEPPE (MALLEE GROWTH FORM) ON ROCKY SOILS

(Contact: Pauline Grierson, University of Western Australia)

Belowground biomass of *Eucalyptus leucophloia* (snappy gum) growing on rocky outcrops in the Pilbara region of north Western Australia was estimated using a combination of excavation and fractal techniques. Individual trees were excavated using a backhoe. Because the sites were extremely rocky, excavations were to as great a depth as feasible, generally between 50 cm and 2 m. Excavations were not separated by depth increments owing to the difficulties of determining depth after removal of large boulders and rocks. Sampling of individuals was stratified by DBH and maximum crown dimensions.

The steps used for each individual excavation were as follows:

- Individual trees were excavated with a backhoe and shovels along one side of the root system (a small trench);
- Once the major roots and taproot or lignotuber were exposed the backhoe was used to lift the lignotuber and major roots and loosen them from the soil and boulders;
- As many major lateral roots as possible were traced out from the lignotuber for as long as possible, generally 2 m. Other lateral roots were severed at about 1 m distance in order to extract the lignotuber, taproot and associated laterals;
- The backhoe was then used to extract the lignotuber from the excavated hole;
- All first and second laterals were measured for their proximal diameters;
- For every first order lateral, root diameters were also measured at regular intervals (5, 10, 20, 50 cm, depending on root length) to determine taper functions for individual roots;
- A sketch and photograph of the excavated root system was obtained. (Figure A2.3);
- Individual lateral roots were harvested to derive basal diameter/root volume to root biomass functions;
- Occasional sampling of roots 0.5-1 cm basal diameter were used to calculate consistency of taper for finer roots and estimate diameter/biomass relationships;
- The site was sampled to estimate fine root biomass by excavation of standard volumes or scoops (with backhoes); and
- All roots were measured for fresh weight and representative subsamples dried for conversion to total root dry weights and for carbon analysis.

Figure A2.3 Lignotuber of Eucalyptus leucophloia **growing on a rocky site in the Pilbara, Western Australia.**

A2.4 EUCALYPTUS WOODLAND COMMUNITIES IN CENTRAL QUEENSLAND

(Contact: Bill Burrows, Queensland Department Primary Industries, Rockhampton)

Allometric relationships for predicting root biomass from stem circumference measured at 30 cm above ground level were developed for 3 different eucalypt species in woodlands in Central Queensland. These relationships were then applied to survey data (stem densities and size) in order to estimate biomass at the community level.

Stand tables (assessment data) were established before commencing root sampling. Then main or "core" plot for sampling was usually within 5 x 100 m transect bands spaced 25 m apart, i.e. the sampling area or "plot" was 100 m x 100 m. Coarse root regressions were established by excavating trees in the external buffer area to the core plot. First, biomass of the lignotubers and main lateral roots was estimated for individual trees by excavation methods. A back hoe or front end loader was used to excavate the lignotubers and larger lateral roots of 10 individuals each of 3 species (*Eucalyptus crebra, E. melanaphloia* and *E. populnea*). Excavation volumes were adjusted according to the size of the lignotuber but had a minimum volume of 1 m^3. Individuals harvested were representative of the range of stem circumferences measured across the study site. Allometric relationships for predicting lignotuber biomass from stem diameters were then developed and applied to the survey data.

Biomass of smaller roots at each site was estimated by coring to 1 m depths. Sampling was stratified within and outside of canopy zones while ensuring a representative cover was obtained of the plot on which the stand table was derived for each species. The approximate canopy cover in the core plot was estimated from the stand assessment data. For

example, if it was 35%, then the "fine root" cores (including all roots regardless of size) were distributed over the plot with 35% under canopies and 65% in inter-canopy zones. Between 40 and 60 cores were collected for each woodland type. Samples were collected using a 120 cm steel tube with an internal diameter of 4.35 cm. Each soil core was sectioned in to five depth intervals (0-20, > 20-40, > 40-60, > 60-80 and > 80-100 cm), which were then washed and sorted into living and dead material (only the live roots were retained).

A2.5 FURTHER CONSIDERATIONS

A2.5.1 TAPER FUNCTIONS AND FRACTAL MODELS

Fractals describe natural structures with self-similarities at different observational scales (Mandelbrot 1983). For root systems, this means that the overall branching patterns may be divided into subsections that have approximately the same appearance as the overall system. Fractals are most useful when the self-similarity is high and for root systems this is dependent on the number of branching orders. This is in turn dependent on environment and genotype. Fractal models have been applied to tree root systems on a number of occasions, most notably by van Noordwijk and colleagues (eg. van Noordwijk *et al*. 1991; Spek and van Noordwijk 1994; van Noordwijk *et al*. 1994) but studies have been restricted to a limited number of species.

A protocol for testing of fractal characteristics of root branching and measurement of parameters for predicting total root system size from proximal root diameters has been developed by van Noordwijk *et al*. (1996) and is described in Table A2.2. It is recommended here that, where possible the additional data be collected in order to test the validity of fractal modelling as a way of predicting total root biomass.

Table A2.2 A. Protocol for quantifying diameters of lateral roots. B. Protocol for testing of fractal characteristics of root branching and measurement of parameters for predicting total root system size from lateral root diameters (modified from van Noordwijk et al. 1996**).**

A. Lateral roots

1. Carefully excavate the first part of the lateral roots at the stem base and where they arise from the root crown;
2. Measure the basal diameters of all first order laterals and second order laterals > 0.5 cm. For every first order lateral, measurements of root diameter at regular intervals (5, 10, 20, 50 cm, depending on root length) to determine taper functions for individual roots;
3. Classify each of the lateral roots by their angle of orientation to the soil surface (the horizontal plane); and
4. Calculate the sum of the root diameter squares for roots with a horizontal orientation (angle with the horizontal less than 45°) i.e. $\sum D_{hor}^2$ and vertical direction $\sum vert^2$

B. Test of fractal branching assumptions

1. Expose parts of the branching system by tracing roots from the root crown or taproot. For each branching point where both the previous and subsequent links have been exposed, measure the diameter of each link (halfway from the link or 5 cm from the previous branching point, outside the thickened area around the branching junction).
2. Analyse data by sorting the roots belonging to a common previous link and calculate the α parameter as $D_{before}^2/\sum D_{after}^2$. Then analyse the regression of α and link length on root diameter. If neither of these regressions has a significant slope then the basic assumptions of fractal modelling are met. The average value of α and link length can now be used in allometric equations for total root length and volume or biomass (see van Noordwijk *et al.* 1994). If regressions of regression of α and link length on root diameter has a significant slope then modified equations will have to be developed (see Spek and van Noordwijk 1994).

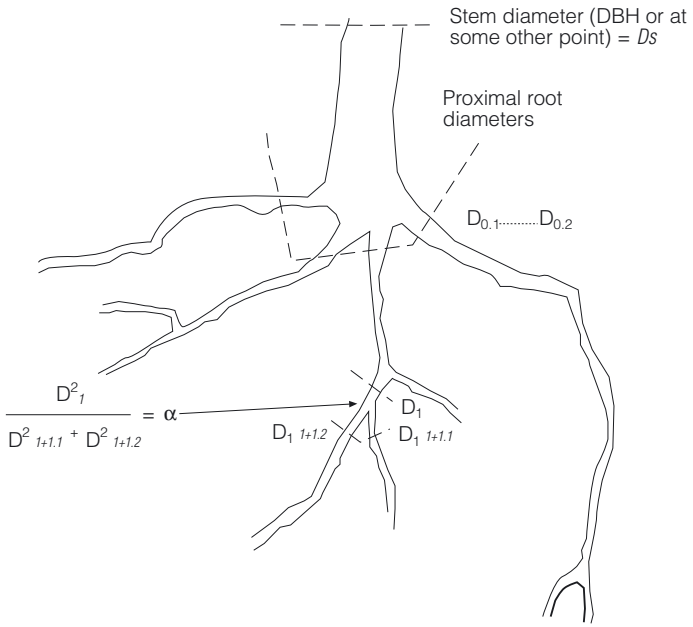

Figure A2.4 Measurement of proximal tree root diameters (modified from van Noordwijk et al. **1996).**

A2.5.2 SPECIAL CONSIDERATIONS

Temporal variation in root growth

Temporal variation in root growth is most important in climates where there is a distinct seasonal difference in temperature or soil moisture content. This leads mainly to fluctuations in the standing biomass of fine roots (< 2 mm). For example, in the jarrah (*Eucalyptus marginata*) forests of Western Australia there can be a three or four-fold difference in total fine root biomass in the 0-20 cm of soil between the dry summer and wet winter months (Grierson and Adams 2001). This amount corresponds to a difference of 5 to 20% of the entire root biomass of the tree (including roots below 20 cm) depending on time of sampling. While the > 2 mm root fraction is unlikely to show significant seasonal variation, the temporal aspects of fine root growth are important for estimating inputs of carbon in to the soil carbon pool from root turnover and are currently not addressed in the soil sampling protocol (McKenzie *et al*. 2001). For these reasons, some additional estimates of < 2mm root fraction are extremely important. Without prior studies it can be difficult to determine exactly when fine root biomass is at a maximum, but patterns of fine root growth will generally follow patterns of water availability. Consequently, sampling for minimum root biomass should correspond with the driest period of the year, for example, in the south west of Western Australia around the end of March. Sampling for maximum fine root biomass should take place when soil moisture availability is greatest, for example, in late September in south west of Western Australia.

Coppice species and lignotubers

The development of predictive relationships between above- and belowground biomass is complicated for coppice systems by the (relatively) frequent replacement of biomass aboveground while belowground biomass remains relatively unchanged or even increases. This is particularly true for mallee types of vegetation where individual lignotubers (a woody swollen structure at the stem base and a type of root crown or root stock) may be up to 2 m in diameter (Jacobs 1955) and there have been reports of individual lignotubers of 75 m^2 in size (Mullette 1978). Repeated stem harvesting or fire can also stimulate the growth of lignotubers, both because the tissue of the lignotuber grows and because the lignotuber merges with the stem base (Canadell and Zedler 1995). Carbon aging of lignotubers of the mallee species *E. oleosa* indicated an age of about 200 years at the centre of the lignotuber while the aboveground stems were only aged in the order of decades (Wellington *et al*. 1979). Consequently, in ecosystems that have a high fire frequency and lignotuberous species, knowledge of site history is essential to interpret data and develop the most robust relationships between above- and belowground biomass.

APPENDIX 3

STATISTICAL METHODS

A3.1 PREAMBLE

Direct estimation of tree biomass is a time consuming, labour intensive and therefore costly procedure. As a consequence methods utilizing ratio and regression techniques have been developed for:

- Estimating biomass of components within trees;
- Estimating total above- or below-biomass from bole dimensions; and
- Relating stand biomass to stand parameters.

These procedures depend on the use of auxiliary variables that are more easily measured but closely related to the variable of interest. Providing sample numbers are sufficiently large, the precision of the regression estimator is better than using a simple mean per unit unless there is zero correlation of biomass with the auxiliary variable. A regression estimate is more precise than a ratio estimate unless the regression forms a straight line through the origin.

Regression methods commonly depend on the relationship of biomass with diameter but a variety of other measures, such as tree height, can also be included. The basal area ratio method (Madgwick 1981) depends upon the relationship between stem sectional area and tree biomass. A special case of the application of ratios is the development of expansion factors whereby stand biomass and carbon content are estimated from timber inventory data for stand volume. The key to the application of these procedures depends on the development of a ratio or regression relationship between biomass and the auxiliary variable in a sample taken from the population of interest. Once it has been established, the relationship can be applied to measurements of the auxiliary variable in the population so that biomass of the population can be estimated.

Mean tree techniques (non-probalistic sample selection) are not recommended for use because there can be no estimate of error nor is it feasible to statistically compare sequential samples (MacDicken 1997).

This section is primarily concerned with:

- The choice of sample trees for destructive harvest;
- The development of regression or ratio estimators for individual trees; and
- Estimation of stand biomass by applying expansion factors.

Details for destructive harvest of sample trees have been given above.

A3.2 CHOICE OF SAMPLE TREES

Biomass studies have been carried out for a variety of purposes, eg. for testing hypotheses about treatments, about stand or ecosystem dynamics, biological processes etc. Such studies are usually carried out in field plots chosen to be representative for the particular purpose. Trees destructively sampled to provide data for developing a biomass estimator are chosen to represent the limited population of interest to the specific study. While this does not preclude the use of the data for other studies any differences in objectives need to be recognized. Biomass relationships developed for users concerned with inventory need to be applicable over a wide range of stand conditions. Two situations arise. If the study is independent of any prior studies, then the trees need to be representative of the entire population to which the relationship is to be applied. This implies that the sample trees will need to be taken from a range of tree sizes within stands of different ages, site qualities and management histories. If data from prior studies is to be included, care must be taken when developing

relationships to avoid bias that might occur by inclusion of data from studies involving a narrow range of, perhaps atypical, stand conditions.

A3.2.1 SAMPLE SIZE

Many biomass studies have used only a small sample (4-12) of trees to estimate the regression relationship between variables. Use of small sample sizes leads to poor estimation of the parameters in the regression equation and to even poorer estimation of the errors involved. The error associated with the estimate of the error mean square (EMS) of the regression will be approximately halved each time the sampling intensity is doubled. However, confidence intervals, which depend on the t-statistic, are not reduced rapidly with an increase in sample size. While relationships based on a small sample of trees may be satisfactory for a discrete population, from 20 to 40 sample trees stratified according to diameter are often recommended for development of generalised tree weight equations for individual species (Clark 1979). Some researchers recommend that 3 sample trees should be selected for every 2.5 cm (Frederick *et al*. 1979) or 5.0 cm (Clark 1979) diameter class. These recommendations seem to be made on an arbitrary rather than a statistical basis (eg. Cunia 1979) but do insure that a large number of trees are sampled and that the full range of tree sizes are sampled.

When a ratio estimate is used with simple random or systematic sampling, determining the number of sample units or trees required to determine a desired confidence interval for the ratio is relatively straight forward provided sample size exceeds 30 so that large-sample working rules can be applied (Shiver and Borders 1996):

$$n = 4 S_u^2 / B_R^2 \mu_x^2 \qquad \text{(A3.1)}$$

where B_R is the chosen half width of the 95 per cent confidence interval, μ_x is an estimate of the population mean of the auxiliary variable, and the variation in the relationship between the auxiliary variable, x, the variable of interest, y, given an estimate of the ratio between them, R, from a sample of size, n, is given by

$$S_u^2 = (\Sigma y^2 + R^2 \Sigma x^2 - 2R \Sigma xy) / n\text{-}1 \qquad \text{(A3.2).}$$

It is often convenient to express the confidence bounds as a percentage, B%. Sample size, n, can then be estimated from the coefficient of variation (CV%):

$$n = 4(CV\%)^2 / (B\%)^2 \qquad \text{(A3.3)}$$

With simple random sampling, estimates of sample size depend only on the specification of the confidence bounds (B) for the results and knowledge of the variance (S^2) of the variable of interest within the population, provided that the population is large. The variance can often be estimated from data from earlier studies or from a pilot survey. For example, in a stand of 100 *Pinus radiata* trees (Forrest 1969) the coefficient of variation of the total above-ground biomass to basal area ratio was 17%. Consequently, 12 trees would need to be sampled to estimate the ratio within +/- 10% of the true value in 95% of possible samples.

A3.2.2 CHOOSING SAMPLES

While random choice of samples can be used, ratio and regression estimators are usually estimated more efficiently if the population from which the samples are taken is stratified (Snowdon 1985, 1992). Strata are often based on some measure of tree size. It is usual to optimize the allocation of samples to each stratum depending on stratum size and variability. Cochran (1977) has discussed guidelines for construction of strata for regression estimators. An approximate rule for constructing sample boundaries so that sample size is constant within strata is to base the boundaries on the cumulant of the square root of the frequency distribution. Unless the variable used to construct the sample boundaries is highly correlated ($r > 0.95$) with the variable to be estimated, there is little advantage in increasing the number of strata beyond 6.

Many studies have shown that above-ground biomass of individual trees is linearly related to DBH raised to a power in the range 2.0 to 2.5 (eg. Snowdon *et al*. 2000). Consequently it is convenient to base stratum boundaries on the cumulant of DBH

distribution. Tree diameters are first listed in order of increasing size. The cumulative sums for each additional tree included in the data set is then calculated. The grand total is divided by the number, N, of strata to be used. The answer gives the cumulative sum associated with the lowest stratum boundaries. The remaining stratum boundaries are determined by successively multiplying the first answer by 2 to N-1. Equal numbers of samples should then be chosen at random from each stratum.

A3.2.3 SUBJECTIVE SAMPLE CHOICE

Many statistical aspects of the development and testing of regression relationships depend upon the assumption that the method of sampling is random in the statistical sense; that is, when for every element of the population the probability of being included in the sample is *non-zero* and *known*, at least in relative terms. Thus, this definition includes various methods used to stratify the population prior to sampling. Measurement of biomass is an expensive operation. As a consequence, it is usual for a degree of subjectivity to enter sample choice. Subjectivity can arise from a number sources. For example, some trees may have a higher probability for acceptance as samples because they are located in situations where the task of sampling is made easier, while other trees may be rejected (i.e. P=0) because they are deemed to be 'atypical' of the general expected form, eg. "wolf" trees, lopsided trees, trees deformed by disease, heavily defoliated, broken topped, or hollow trees. It is typical of biomass studies that the sample trees are generally healthy and vigorous with high quality stems. It is likely that non-random choice of sample trees will lead to under-estimation of the associated errors and may lead to biased estimates of biomass.

A3.3 TREE MEASUREMENT AND CHOICE OF AUXILIARY VARIABLES

Many variables have been used in allometric and linear equations for estimating biomass. Diameter at breast height (DBH) is the most widely used variable. This is often the only variable required if biomass within a single stand is to be estimated because other variables, such as height, are closely correlated with DBH within a given site. However, the relationship between diameter and height often varies over the geographic range of a species depending on site conditions. In these circumstances equations including both DBH and height result in an improved relationship. In allometric equations it is best that DBH and height be used as independent variables rather than as a composite variable (eg. DBH^2 x height) because this allows a more flexible description of the data (eg. Snowdon 1985).

The use of a fixed reference point, such as breast height, for bole diameter measurement can lead to problems with allometric equations particularly for trees less than 5 m tall (Snowdon *et al*. 2000). As a consequence biomass equations may vary with tree age reflecting the change in location of the reference point for diameter measurement relative to tree height. Addition of a small positive constant value to the diameter measurement can substantially correct for measurement-location-induced curvilinearity in allometric relationships (Madgwick 1979). Alternatively, a constant can be included in the equation and the parameters estimated by a non-linear estimation procedure. The best solution is to choose a measurement location closer to the tree base when regression relationships are to be developed for small trees. Whatever the measurement may be, it is important that standard forestry protocols for making those measurements should be used (eg. Anon. 1995, Ellis and Hayes 1997, Wood *et al*. 1999).

A3.4 REGRESSION ESTIMATORS

A3.4.1 REGRESSION FUNCTIONS

Regression functions are equations that relate biomass of vegetation components to auxiliary variables that are easily measured, such as tree dimensions. Calibration data are obtained for biomass and tree dimensions from individual trees that are representative of the population. The

relationship is derived by regression analysis, which can be applied to provide an expected value of the dependent variable (component biomass) for given values of the independent variables (measured tree dimensions). The associated error in the regression relationship can be used in tests of confidence in the predictions provided that assumptions about the statistical distribution of errors hold true.

The usual form of the regression function is:

$$Mi = f_i(X) + \varepsilon_i \qquad \text{(A3.4)}$$

where, M is the mass of the ith component (leaves, branches, stems)

f_i is the chosen regression function

ε_i is the error term

X is the vector of independent variates, such as diameter and height.

In simple linear and multiple linear regression calculated by the ordinary least squares method the line of best fit is estimated so that the sum of the squares of deviations of the observed values from the expected values is minimised. This is appropriate when the errors are assumed to be normally distributed. If they are not then other methods can be used. When the variance of the regression relationship departs in a known manner from the assumption of normality, weighted least squares can be used to estimate the regression line. In this case each observation is weighted by the inverse of its variance. Another common approach is to apply a mathematical function (eg. logarithmic transformation) so that the deviations in the data conform to the assumption of normality.

In some cases the regression function will be nonlinear in some of its terms, eg. the allometric or power function. In some cases these can be linearised by applying a suitable transformation of the data, eg. logarithmic transformation of dependent and independent variables in the case of the allometric relationship. Ordinary least squares can then be used to calculate the line of best fit. Otherwise, nonlinear regression techniques are available to solve the problem.

Regression equations are derived for populations where it is assumed that the geometric relationship between dimension and biomass is constant. Equations are usually site and species specific. The scale at which the equations can be used for prediction will depend on the range of trees sampled to obtain the calibration data, the magnitude of variation within the population, and the degree of uncertainty that is acceptable in the value of predicted biomass. A set of equations is often derived for each of the tree components, i.e. leaves, twigs, branches, stem, and the total, as functions of linear dimensions.

A3.4.2 ALLOMETRIC EQUATIONS

Living organisms exhibit size-correlated variations in form which is referred to as allometry (Huxley and Teissier 1936). The relationship between size and form is based on the premise that size influences the behaviour of structural, mechanical and chemical systems.

In forestry applications, biomass of tree components are related to easily measured auxiliary variables, such as bole dimensions. Allometric relationships are described by using simple power equations. Generally, logarithmic transformations to base *e* have been used to ensure: a) independence of mean and variance; and b) residuals conform to a normal distribution. These criteria are necessary to satisfy assumptions of statistical tests for the linear regression (in logarithmic space) used in allometric methodology. The general form of the power equation is:

$$Y = \beta X^{\alpha} \qquad \text{(A3.5)}$$

where α is the equilibrium constant and β is the size of y when x=1. The special case when $\alpha = 1$ is known as isometry. The relationship is often used in its log-transformed form:

$$\ln (Y) = \ln (\beta) + \alpha \ln (X) \qquad \text{(A3.6)}$$

A3.4.3 BIAS AND NON-ADDITIVITY

When logarithmic, or indeed any transformation is made to the dependent variable, the back-transformed mean will be biased. For common transformations, formulae are available to correct the bias. Some of these are mathematically exact but others, such as the correction for bias after logarithmic transformation, depend on estimation of other quantities and/or assumptions about the distribution of statistical errors in the data. The corrections used with logarithmic transformations (eg. Baskerville 1972, Beauchamp and Olson 1973, Flewelling and Pienaar 1981) depend on the estimation of the variance of the relationship and on the assumption that the errors are normally distributed in the log-transformed space. In most allometric studies of forest biomass there are insufficient data to reliably estimate the variance while the distribution of errors is unknown although it may be approximately normal.

The method that is recommended when the original calibration data is available is the ratio method (Snowdon 1991). This is a robust method, free from the limitations noted above, and is based on direct estimation of bias in the sample:

$$\text{Estimate of true biomass of population} = \frac{\text{True biomass of sample}}{\text{Estimated biomass of sample}} \times \text{Estimated biomass of population}$$

where the estimates are made by application of an allometric relationship.

When various component weights, i.e. stem, branches, foliage, are estimated using log-transformed allometric relationships, they do not sum to the same total that is obtained when the total is estimated directly by an allometric relationship. Many studies do not provide equations for total above-ground biomass. Thus, any attempts to estimate total biomass by addition of estimates of the components will, by necessity, be biased.

A3.4.4 NON-LINEAR ESTIMATION

The parameters in the allometric equation, $Y = \beta X^{\alpha}$, can be solved by using non-linear regression techniques. This method avoids the bias inherent when the independent variable is transformed prior to analysis. The equation can also be extended to incorporate an intercept (χ):

$$Y = \chi + \beta X^{\alpha} \qquad \text{(A3.7)}$$

The intercept can be used to account for the small biomass of trees that have zero diameters at the point of measurement. A weighted least squares can be used to overcome heteroscedasticity of variance. Variances for woody components are commonly proportional to the square of diameter (D), i.e. the appropriate weighting factor is $1/D^2$. This can be used as a first approximation but generally a method such as that described by Bi and Hamilton (1998) should be used.

A3.4.5 LINEAR REGRESSION

Simple regression estimators are used when the relationship between the variable of interest and the auxiliary variable is linear but does not necessarily pass through the origin of the x:y coordinate system. The regression estimator is a biased estimator but the degree of bias is negligible when the true relationship between the variables is linear.

When stand biomass (ΣY) is estimated from the application of a linear equation for predicting tree biomass (Y) from simpler tree measurements (X_i), eg.

$$Y = \beta_0 + \beta_i X_i \qquad \text{(A3.8)}$$

then a corresponding stand based linear equation exists:

$$\Sigma Y = N\beta_0 + \beta_i \Sigma i \qquad \text{(A3.9)}$$

where N is the number of trees in the stand and β_0, β_i are coefficients in the model. Such a simple relationship does not exist when non-linear methods such as the allometric equation are used.

One of the main characteristics of the equations that should be considered is the form of the curve beyond the size range of the data. Polynomial prediction equations, for example, can produce unusual results when extrapolated. Functions with more conservative forms and slopes are preferable. It is best to restrict the application of these equations to the data range used for their calibration.

In contrast to allometric equations, methods are available which ensure that the estimates of different components sum to a consistent estimate of the total (Cunia and Briggs 1984, 1985).

Weighted regression can be used to overcome problems arising from heteroscedacity of errors.

A3.5 RATIO ESTIMATORS

Ratio methods depend on estimating the ratio the variable of interest, eg. tree biomass, and a more easily measured auxiliary variable. In general terms the method is applied as follows:

$$\text{Total biomass of population} = \frac{\text{Total biomass of sample}}{\text{Total for auxiliary variable in sample}} \times \text{Total for auxiliary variable in population}$$

This ratio of means estimator is a best linear unbiased estimator if the true relationship between the two variables is a straight line through the origin and the variance of the dependent variable about this line is proportional to the independent variable (Cochran 1977). For above-ground biomass the ratio is often formed between tree biomass and bole basal area (Madgwick 1981) and is known as the basal area ratio method. This is easily applied to basal area estimates obtained from variable radius plots. Snowdon (1992) has developed a modified method in which the relationship can more closely approximate a power curve. However, since the auxiliary variable contains a nonlinear component, the method is not as easily applied to point estimates of basal area.

If the auxiliary variable is derived from a measure of bole diameter taken at a fixed reference point, eg. at 1.3 m, then inaccuracies in prediction will occur with small trees.

A3.6 EXPANSION FACTORS

Commonly, stand- or forest-level estimates of total stemwood volume, merchantable wood volume or basal area are available for forests where timber is harvested. In these cases the most efficient estimate of biomass may be derived from application of wood density and expansion factors to stand inventory data (eg. Brown 1997).

Biomass density of a stand (B_{stand} in t/ha) can be estimated from:

$$B_{stand} = V*D*BEF \qquad (A3.10)$$

where

V = some measure of stem volume (m^3/ha);

D = wood density (t/m^3); and

BEF = biomass expansion factor.

Stemwood volume. Generally Equation A3.10 is only applied if stemwood volume data are already available. Various measures of stem wood volume may be used including total stemwood volume (gross bole volume) and merchantable volume (volume above a stump allowance and below a nominated small-end diameter limit). Usually the volumes will be under-bark (wood only) but may be over-bark (wood plus bark). Obviously the appropriate values for D and BEF in Equation A3.10 will depend on the measure of stemwood volume used.

Wood density (D). As commended by Brown (1997): 1) The appropriate value for D in Equation A3.10 is volume-weighted average wood density, based on dominance of each species as measured by volume; and 2) If data for all species are not known, it is best to estimate weighted average wood density using species-specific estimates for known species and regional averages for other species.

Biomass expansion factor (BEF). Where the biomass (*B*), stem volume (*V*) and stem density (*D*) of individual sample trees have all been estimated from the methods outlined in this Section 3 the *BEF* can be estimated by re-arranging Equation A3.10:

$$BEF = B/(V*D). \qquad (A3.11).$$

Note that both density and the biomass expansion factor are ratios. Consequently, the remarks made above regarding sample sizes etc. for ratio estimators should also be applied to these estimators.

A3.7 STAND LEVEL ESTIMATES

In many biomass studies the populations of interest have been restricted to relatively small plots where it has been feasible to measure the auxiliary variable on all trees. When it is impractical to measure the entire population, double sampling or two-phase sampling can be used. In the first phase a set of sample plots are established in order to estimate the value of the auxiliary variable in the population. In the second phase a sub-sample of trees or plots is used to estimate the relationship between biomass and the auxiliary variable. Sometimes an estimate of the relationship derived from an independent study is used. In this situation, the equation used to calculate variance should recognize that the two phases are independent.

A3.7.1 CHOICE OF SAMPLE PLOTS

Estimates of forest biomass can often be made by applying relationships as developed above to tree measurement data from existing plots. In many cases new plots will need to be established and new tree measurements taken. The sampling design for a series of new plots will usually be prepared by an expert in forest inventory.

Simple random sampling is not often used as a sampling design because particular samples (i.e. collections of plots) may not be representative of all parts of the population. Prior knowledge of the population can be used to increase the precision and efficiency of the sampling design by grouping sub-populations into strata which can be sampled independently. If within-stratum variation is less than between-strata variation then the estimate of the total population will be more precise than with simple random sampling. At least two plots must be taken from each stratum.

Systematic selection of plots for inclusion in a sample is often a cost effective alternative to simple random sampling, however, the probabilities of different sets of units being included in the sample are not all equal. Provided there are no periodic features in the population, a systematic sample with a random starting location will provide a more accurate estimate of population parameters than a stratified random sample of the same size. If there are unrecognized periodicities in the population it is possible to get very biased estimates. In many cases systematic sampling allows a spatial distribution map to be easily constructed. Systematic selection is often used as the last stage of a multi-stage sampling procedure. Often this will involve the systematic allocation of a line or grid of quadrats or plots.

When establishing plots it is important to be aware of edge and other effects on tree growth when they are located near population boundaries or near areas of disturbance such as roads, old skid sites, logging tracks, gaps, windblown areas etc. From an inventory point of view, plots that fall into these areas should be retained if the affected area is counted as part of the stand area but not if it falls within a void that is not counted towards stand area. A subjective choice may be made to exclude affected trees from the sample used to estimate the regression relationship (but see Section A3.2.3). This can be accomplished by ensuring that sample plot boundaries are distant by more than an average tree's height from the stand boundary or area of disturbance. Plots in which destructive sampling is to be carried out should not be located in areas, such as stream reserves, in which harvesting is prohibited.

A3.7.2 TYPES OF SAMPLE PLOTS AND POINTS

Plots of various kinds form the basis of most inventory systems. Temporary plots, which are measured only once, may be used in many circumstances. Permanent plots, which are measured on several occasions, are more expensive but are essential when change is to be measured. The proportions of temporary and permanent plots used will depend upon the purpose of the inventory.

Fixed area plots

The use of fixed area plots is the oldest and most widespread method of sampling in forests. Much effort has been placed on determining the plot shapes which minimize variance under various stand conditions but square, rectangular or circular plots can all be used with confidence if care is taken in determining which trees are included in the plot. Circular plots are often preferred because of ease of establishment and because boundary effects are minimized. Square and rectangular plots may be easier to establish on steep slopes and are still commonly used in systematic strip surveys.

Multi-area plots

This system of sampling, sometimes referred to as multiple radius sampling, has been promoted as an efficient method for measuring uneven-aged stands. It is an equal probability design that should not be confused with variable probability sampling designs associated with variable radius plots. In uneven-aged stands large plots are required to adequately sample the larger size classes, but, if large plots are used the number of small trees to be measured is also large. The solution to this problem is to define a series of plot sizes each of which samples a different part of the size range. In N.S.W., a set of three circular, concentric plots are used (Carter 1994). The inner 0.1 ha plot samples trees 10.0-29.9 cm diameter at breast height over bark (DBHOB), the 0.3 ha plot samples trees 30.0-49.9 cm DBHOB while the 0.6 ha plot samples trees of 50.0 cm DBHOB.

A more complex arrangement of plots is utilized for national resource inventory in the U.S.A. (Spencer and Czaplewski 1997). The current system uses a cluster of four sets of multi-area plots within a one-hectare circular plot.

Variable radius plots

Horizontal variable radius plot (VRP) sampling (Bitterlich 1947) was introduced in forestry as a quick, low cost method of obtaining estimates of stand total basal area by a simple counting technique. The procedure is also commonly called angle count sampling or point sampling (Schreuder *et al*. 1993). During an assessment an observer visits a number of points in the forest stand and with the aid of an instrument, commonly a wedge prism, counts the number of trees that subtend a greater angle than the fixed critical angle generated by the instrument. The inclusion probability is proportional to tree diameter, i.e. the method is an example of probability proportional to size (PPS) sampling. The number (n) of trees counted is multiplied by the basal area factor (BAF) of the instrument to give an estimate of the stand basal area (G, m^2ha^{-1}). In fully stocked stands BAFs of 2 to 5 are appropriate in most situations, but, in heavily thinned stands and young poorly stocked forests BAFs of 1 to 2 would be more appropriate (Wood *et al*. 1999). The first stage of Victoria's Statewide Forest Resource Inventory (SFRI) is based on VRP sampling with a BAF of 3 which gives an average of 10-15 trees per sampling point (Hamilton *et al*. 1999).

A3.7.3 PLOT SIZE

Plots should be sufficiently large to contain 20 or more trees. The number of plots required will depend on the range of conditions which need to be sampled within the nominated population. Plot size becomes particularly important if the plots are to be used to estimate area dependent stand parameters in addition to characterization of the size distribution of the trees. Plot area should always be specified on a horizontal plane. If the average slope is less than about 10° measurement of plot area can be achieved by ensuring that the measurement tape is held horizontally. Otherwise suitable slope correction factors should be applied (eg. Ellis and Hayes 1997, Appendix 4). If the plots are to be revisited it is important to mark the plots for easy identification and to record and/or map their position so that they can be easily relocated.

A3.7.4 ESTIMATION OF BIOMASS

In each plot each tree is measured for the auxilliary variables used to develop the relationship with biomass, eg. DOB, tree height, merchantable volume. The relationships are then applied to the mensurational data to obtain an estimate of tree biomass. The sum of biomass for all trees in the plot then provides an area-based estimate of stand biomass. Depending upon the inventory design, these plot based estimates can be combined to obtain estimates of biomass at compartment, forest and regional levels of aggregation.

A3.7.5 ERROR ESTIMATES

Whenever a regression or other relationship is used to predict biomass an estimate of error should also be given. This is straight forward when a simple inventory design has been used but can be complex for more sophisticated designs used for inventory. For example, there are two main sources of error in the estimates of average above-ground biomass of overstorey per hectare:

- The error due to variation between tree diameters (or other auxilliary variables) within the forest. The size of this component depends on sampling design, sample size, type of estimator and inherent variation between sample units (Cunia 1987); and
- The error of the biomass regression (or ratio) which is also affected by sampling design, sample size, estimation procedure and inherent variation in biomass values about the regression line.

It has been common practice in forest inventory to ignore all sources of error except the first, it being implicitly assumed that the error due to the biomass regression function is small and can be ignored (Cunia and Michelakackis 1983). The main reason for this attitude was that for a long time forest biometricians did not know how to deal with the problem. In the case of weighted linear regression where the first and second stage samples can be regarded as independent an estimate of the variance of the biomass estimate ($Var\ (w)$) can be obtained :

$$Var\ (w) = b' S_{zz}\ b + z'\ S_{bb}\ z \qquad \text{(A3.12)}$$

where b and z are vectors containing the regression coefficients and S_{bb} and S_{zz} are their associated covariance matrices (Cunia 1987). In worked examples Cunia (1987) found that the variance for the estimated biomass was underestimated by 33 to 54 per cent when the contribution due to the error in the biomass regression function was ignored.

Estimates of error associated with estimates for each compartment of carbon submitted in the report should be made. These will depend on the particular field methods used to estimate each component. Since the variances of the carbon content of the different compartments are unlikely to be independent it is not feasible to sum the variances to estimate an overall error term. The most practical way to calculate overall error is to estimate total carbon content (i.e. the sum of the various compartments) on a series of replicated plots, and to then use the variation between plots as an estimate of total error.

APPENDIX 4

SLOPE CORRECTIONS FOR PLOT RADII AND DIAGONALS

Table A5.1 Slope correction for plot radii (m) of circular plots measured on a slope needed to obtain nominated area in the horizontal plane.

Slope	Plot area m²							
	100	250	400	500	667	800	1000	2000
0	5.64	8.92	11.28	12.62	14.57	15.96	17.84	25.23
5	5.65	8.94	11.31	12.64	14.60	15.99	17.88	25.28
10	5.69	8.99	11.37	12.71	14.68	16.08	17.98	25.43
15	5.74	9.08	11.48	12.84	14.83	16.24	18.15	25.67
20	5.82	9.20	11.64	13.01	15.03	16.46	18.40	26.03
25	5.93	9.37	11.85	13.25	15.31	16.76	18.74	26.50
30	6.06	9.59	12.13	13.56	15.66	17.15	19.17	27.11
35	6.23	9.86	12.47	13.94	16.10	17.63	19.71	27.88
40	6.45	10.19	12.89	14.41	16.65	18.23	20.38	28.83
45	6.71	10.61	13.42	15.00	17.33	18.98	21.22	30.01

Table A5.2. Slope correction for plot half diagonals measured on a slope needed to obtain nominated area in the horizontal plane.

Slope	Plot area m²							
	100	250	400	500	667	800	1000	2000
0	7.07	11.18	14.14	15.81	18.26	20.00	22.36	31.62
5	7.10	11.22	14.20	15.87	18.33	20.08	22.45	31.74
10	7.18	11.35	14.36	16.06	18.54	20.31	22.71	32.11
15	7.32	11.57	14.64	16.37	18.91	20.71	23.15	32.74
20	7.52	11.90	15.05	16.83	19.43	21.28	23.80	33.65
25	7.80	12.34	15.60	17.45	20.15	22.07	24.67	34.89
30	8.16	12.91	16.33	18.26	21.09	23.09	25.82	36.51
35	8.63	13.65	17.26	19.30	22.29	24.42	27.30	38.60
40	9.23	14.59	18.46	20.64	23.84	26.11	29.19	41.28
45	10.00	15.81	20.00	22.36	25.83	28.28	31.62	44.72

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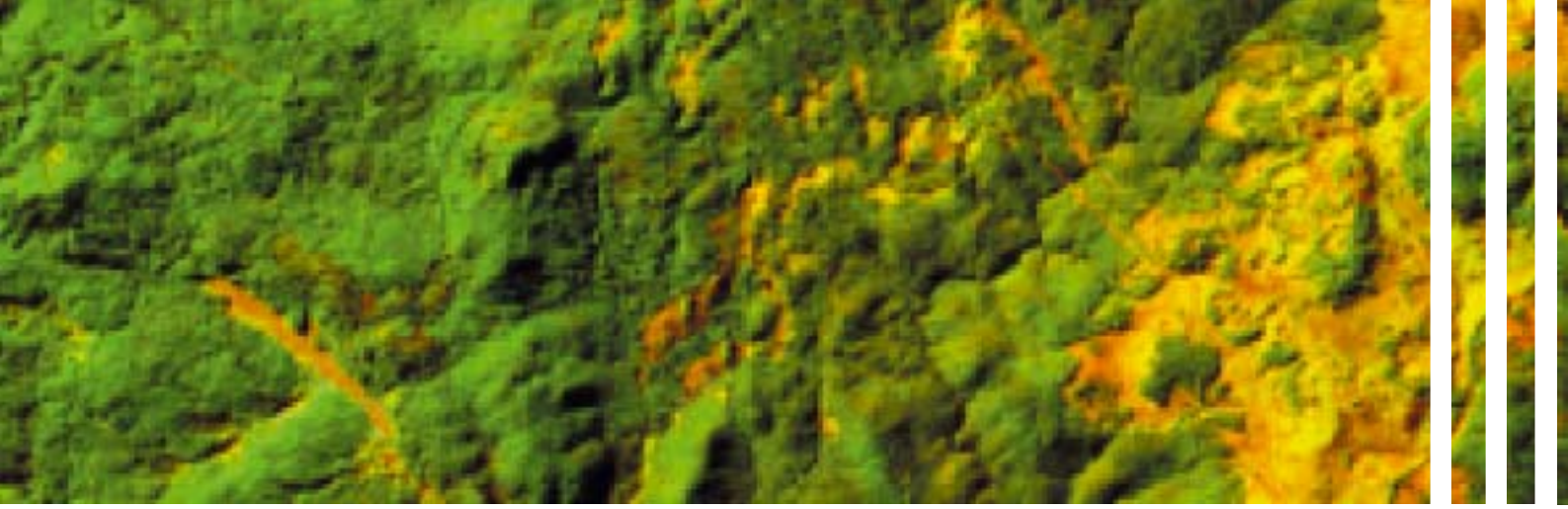

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