Seasonal and spatial variations in composition of phloem sap of *Eucalyptus globulus*, and other factors that predispose plantations to attack by *Barnardius zonarius* parrots in the south-west of Western Australia

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Statement of original work

I declare that this thesis comprises all my original work, other than where specifically acknowledged, and that it is not substantially the same as any other thesis that has been submitted at any other University.

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Frontispiece – Port Lincoln parrot (*Barnardius zonarius zonarius*) in Marri (*Corymbia calophylla*), its favoured habitat tree. In south-western Western Australia, this subspecies hybridises with *B. z. semitorquatus*, and both subspecies and their hybrids are referred to as 'Twenty-eight' parrots. (Photo reprinted with permission from Agriculture Western Australia).

ABSTRACT

Port Lincoln parrots (*Barnardius zonarius zonarius*) are severely damaging many young plantations of Tasmanian bluegums (*Eucalyptus globulus*) in the south-west of Western Australia. These parrots strip the bark from lead shoots when feeding on soft cambial tissues and phloem sap, thus leading to trees being deformed and difficult to harvest. This study investigated the distribution of parrot damage, and identified several factors that explained variability in damage within and between plantations. The effects of soil water and nutrient availability on tree growth and phloem sap composition were also assessed to determine whether such site factors influenced tree selection by parrots.

Damage was surveyed in 465 plots, each of 15 trees, of two-year-old *E. globulus* selected from 12 plantations across the south-west of Western Australia, where mean annual rainfall ranges from 650 - 1430 mm. The proportion of trees damaged per plot varied among plantations from zero to 73 % of trees. More than one third of all trees surveyed were damaged, and the mean length of bark stripped on main stems per plot was 10.6 cm. Trees with adult stems and foliage were damaged more frequently and severely, than those still in juvenile stage.

Differences in damage among plantations were strongly related ($r^2 = 0.76$) to estimates of parrot abundance. Damage was greater in lower rainfall, cereal growing regions, than in high rainfall central and southern regions. Damage within plantations decreased with increasing distance in from plantation edges, and with increasing distance from the nearest mature native trees. Plantation trees adjacent to linear remnants of native vegetation, or to scattered paddock trees, experienced almost twice the damage of those adjacent to state forest, or to another plantation. Where there was a relatively even distribution of parrots surrounding plantations, damage was positively related to tree growth ($r^2 = 0.98$), as measured by diameter of main stem at breast height. A Generalised Linear Model was developed which explained 70 % of the variation in damage encountered among plots.

Seasonal and spatial variations in phloem composition were examined in 29 plots in three plantations of one to two-year-old *E. globulus*, encompassing a wide variety of soil types and mean annual rainfall. Variations in stable carbon isotope ratios in phloem sap were used as a novel indicator of water stress. $\delta^{13}C$ values varying broadly from -27 ‰ to -19 ‰, and were least negative during autumn, in low rainfall regions, and in trees growing in shallow soils. Tree growth was also strongly related to phloem $\delta^{13}C$ values ($r^2 = 0.68$) in one plantation, whereas in others, growth was affected by poor nutrient availability.

Phloem sap contained high concentrations of total sugars (20 - 30 % w/v), predominantly sucrose and raffinose. Total amino acid concentrations varied from 0.7 - 24 .2 μmol ml^{-1}, with glutamine accounting for most variation. Major organic acids were malic, citric, shikimic and fumaric. Concentrations of sugars increased linearly with increasing water stress (phloem $\delta^{13}C$) ($r^2 = 0.70$), regardless of tree nutrient status, and were negatively related to tree growth. Conversely, total amino acid concentrations were positively related to tree growth ($r^2 = 0.53$), and decreased markedly in trees affected by seasonal water stress. Total C:N ratios in phloem sap therefore varied widely (200 - 8200), and were negatively related to tree growth ($r^2 = 0.64$).

During autumn, the season of most severe damage to trees, parrots selected faster growing trees with greater concentrations of total amino acids in phloem sap and greater phloem sap volume, but lower concentrations of total sugars. Analysis of phloem sap collected at different heights up the stem showed amino acid concentrations to increase to a maximum in uppermost regions where stems were typically most damaged. By contrast, sugar concentrations decreased with stem height. So, while parrots would undoubtedly benefit from the sugars in phloem sap, their selection of trees during autumn appears related to tree growth and nitrogen status, and other, as yet, undescribed factors. The possibilities that parrots may seek cambium for its protein or phloem sap for its water are discussed.

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 The Tasmanian bluegum (*Eucalyptus globulus*) industry in Western Australia

Eucalyptus globulus subsp. *globulus* is one of the major eucalypt species currently grown in plantations in eastern Victoria, northern Tasmania and south-western Western Australia for the production of high quality hardwood fibre and timber. The species occurs naturally in coastal regions of southern and eastern Tasmania, and to a lesser extent in southern Victoria, south of latitude 30° S and below altitudes of 400 m. There it favours areas with a moderately high rainfall (500 mm - 1500 mm mean annual rainfall), moderate summer temperatures, and good quality heavy soils or loams (Millett, 1969). Despite its restricted natural range, *E. globulus* is grown successfully in many countries around the world with vastly differing soil and climatic conditions, including Portugal, Spain, South Africa, India, China, Chile and Columbia. It responds well to intensive silviculture (weed and insect control, fertiliser application) and may grow rapidly on favourable sites (Cromer *et al.*, 1981; Cromer and Williams, 1982; Schönau and Herbert, 1989; Nambiar, 1990; Tomé and Pereira, 1991; Judd *et al.*, 1996).

In Western Australia, an estimated 120,000 ha has been planted (October, 1999), mostly in the last nine years, with a current increase of about 20,000 ha per year. Most plantations are on farmland, which had previously received annual dressings of phosphatic fertiliser. Plantations will be harvested for woodchips after nine to ten years. Mean annual increments of stem volume (MAI) up to 40 $m^3 ha^{-1}$ are possible on deep soils in areas with an annual rainfall exceeding 1000 mm. The plantable area for woodchip production is limited mainly by annual rainfall (>600 mm), soil depth (>2 m), and in economic terms, by haulage distance (<100 km) to the nearest woodchip mill. Annual rainfall varies from 600 mm to 1400 mm across the plantable area, decreasing from south to north and from west to east (Fig. 1.1). The rainless 'summer' period in this region with a Mediterranean climate can last up to six or seven months in drier areas, significantly increasing the risk of *E. globulus* dying from drought (Hingston *et al.*, 1998).

Soil type and soil depth vary greatly in south-western Australia (Mulcahy, 1973; McArthur, 1991; Churchwood, 1992). Deep lateritic profiles of ironstone gravel over kaolin clay up to 30 m deep are widespread on the undulating Darling Plateau, which rises abruptly in the west above the Swan Coastal Plain, and extends from north of Perth to Mount Barker in the south. As a result of uplifting and dissection of the Darling Plateau, younger, more fertile soils have developed along deeply incised valleys, associated with major river systems, many of which are quite shallow to bedrock. Along the west and south coasts are deep coarse sands derived from aeolian or fluvial deposits. Variations in soil type and soil depth can be found over relatively short distances and markedly affect the availability of water and nutrients for tree growth. Considerable numbers of trees have died in plantations of *E. globulus* (Dutkowski, 1995; Hingston *et al.*, 1998) and *Pinus radiata* (McGrath *et al.*, 1991) in the south-west, even in the higher rainfall regions, where soil depth (defined as depth to rock or saprolite) was inadequate to store sufficient soil water over summer.

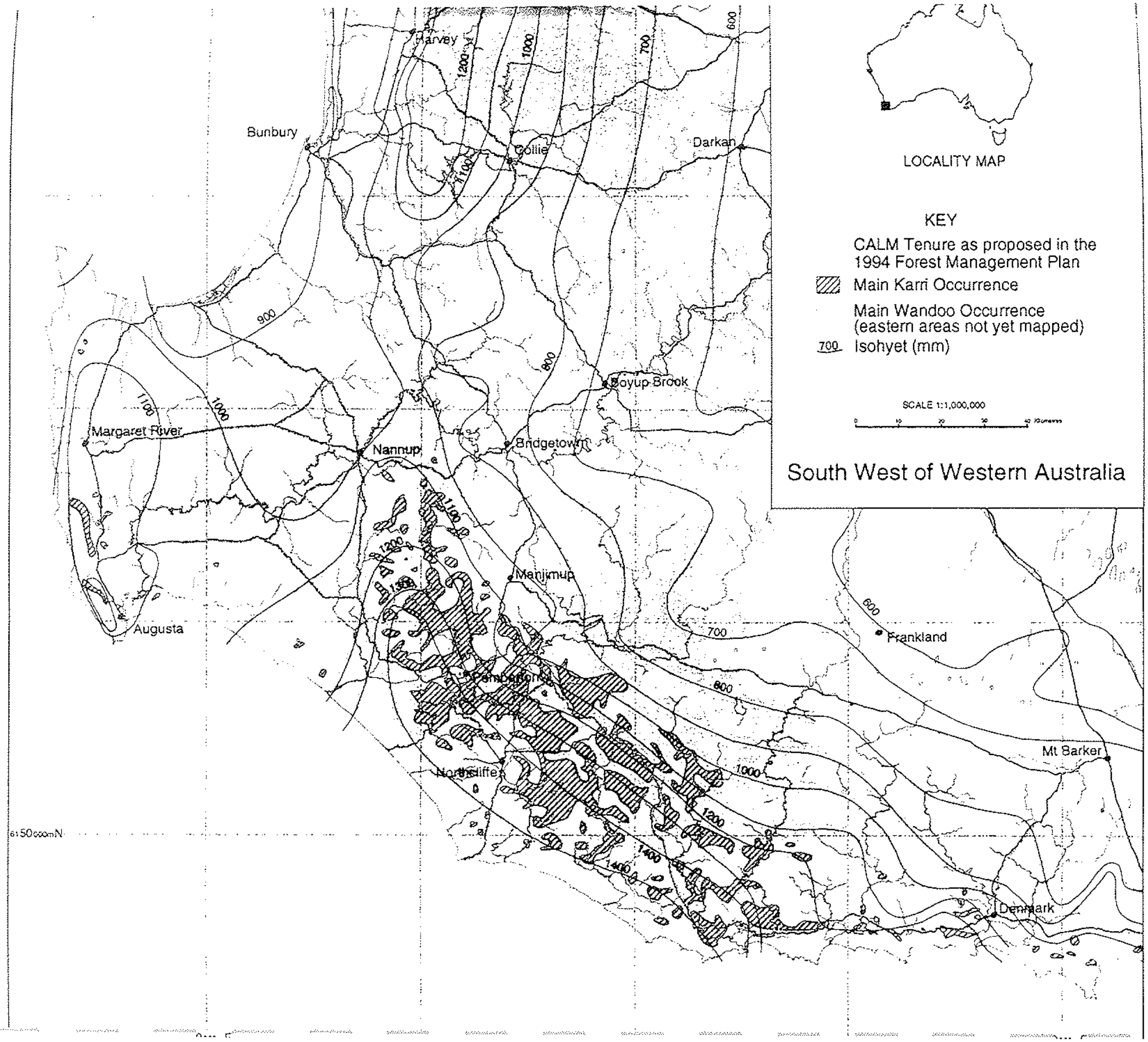


Figure 1.1 Location map of south-western Western Australia showing rainfall isohyets, major towns and areas of native vegetation (Department of Conservation and Land Management).

Figure 1.2 Parrot damage in 18-month-old *Eucalyptus globulus* showing bark-stripping on central lead shots and some lateral shoots. Most damage is found where the stem diameter is less than 2 cm, and within 2 m of the stem apex.

Figure 1.3 The result of earlier parrot damage in eight-year-old *Eucalyptus globulus*. Repeated attacks can cause multi-stemmed trees with severe stem deformities that are difficult and costly to harvest.

Figure 1.4 Parrot damage on six-month-old seedling of *Eucalyptus globulus* showing characteristic scraping marks on the cambium made by the parrot's beak. The bark is stripped off the stem to reveal the cambium and discarded.

1.2 Parrot damage to *Eucalyptus globulus* plantations

Ringneck parrots (*Barnardius zonarius*) may severely damage young plantations of *E. globulus* (and other eucalypt plantings) in the south-west of Western Australia by stripping the bark on lead shoots (Fig 1.2). From personal observations in the field, and in aviary trials (see pp 112), parrots initially loosen a strip of bark vertically from a stem with their beak. They then hold a section of bark in one hand and appear to scrape the phloem with their lower mandible from inside the strips of bark. The bark itself is not chewed and is soon discarded (Fig. 1.4). Finally, they use a transverse scraping action across the stem to feed on the cambium and any adhering phloem sap.

Complete removal of bark from around a stem, called 'ring-barking', usually results in forked and deformed trees (Fig.1.3) which are difficult and costly to harvest and process. One estimate of the cost of lost production owing to parrot damage was \$150 ha^{-1} yr^{-1} (R. Breidahl, Bunnings Treefarms, pers. comm.). In extreme cases, plants only 1m high can be ring-barked at ground level leading to the death of the tree (Fig 1.4). Stem deformities are also caused by other factors (e.g. wind breakage, copper deficiency, sheep grazing) but these can be readily distinguished from parrot damage.

Barnardius zonarius belongs to the family Psittacidae, the broad tailed parrots. The nominate subspecies of *B. zonarius* is *B. z. zonarius* (Port Lincoln Parrot). Its range extends from Eyre Peninsula in South Australia to the Northern Territory border, west to the Pilbara region and south to the central regions of Western Australia. It lives in semi-arid eucalypt-dominated woodlands in the wheatbelt, in arid eucalypt or mulga scrublands of the central regions, and in woodlands along rivers and watercourses in dry spinifex country (Serventy and Whittell, 1967; Forshaw and Cooper, 1981). *Barnardius z. semitorquatus* (Twenty-eight Parrot) is a subspecies said to be confined to the heavy forests of south-western Western Australia, west of a line from Perth to Albany (Forshaw, 1969). The two subspecies hybridise where their ranges overlap and intermediate forms are common, particularly in the wheatbelt (Serventy and Whittell, 1967). A further subspecies, *B. z. occidentalis*, inhabits north-western Australia. As a result of hybridisation, there is much confusion in the literature regarding the status and description of the species and its races (Forshaw, 1969; Crome and Shields, 1992). To add to the confusion, all varieties of *B. zonarius* in Western Australia, irrespective of plumage, are known as 'Twenty-eights'. In this thesis I will use the terms 'parrots' and 'parrot damage' to refer specifically to the subspecies *B. z. zonarius*, *B. z. semitorquatus*, and their hybrids which appear to be solely responsible for the current damage to eucalypt plantations in south-western Australia (see Frontispiece).

Plantation-grown *E. globulus* normally grow with strong apical dominance and a single stem. The section of stem most affected by parrots is typically 1.0 - 2.0 m from the top of the tree, where the stem diameter is 0.5 - 2.0 cm. Stems that have been stripped or ring-barked are weakened and frequently break during strong winds, before initiated wounds can heal over. The loss of the lead shoot causes one or more lateral shoots from below the wound to replace the leader, but these can also be damaged (Fig 1.5). Double or multi-stemmed trees result with bends or forks that can render the log unacceptable for harvesting operations. Trees of any age are attacked, but damage to young trees undoubtedly causes greatest commercial loss (Ritson, 1995).

Ritson (1995) identified a 'Critical Period' during which parrot damage needed to be prevented in plantations, as the time from planting until trees were old enough to produce logs of minimum length to meet harvesting and processing criteria. The minimum length for chipping is 2.0 m, but 6.0 m is preferred for logging and carting operations. The 'base' log (from the base of the tree) is the most valuable since it has the greatest volume. In average growing conditions, approximately 2 years from planting is required to produce a base log so that protection from parrots during this time is of highest priority. Critical Periods for *E. globulus* plantations on drier sites are longer because trees will take more time to reach minimum suitable log length. Damage after this time will still result in loss of volume, but will be less significant economically.

Figure 1.5 Parrot damage caused early breakage of the central lead shoot, which resulted in a side branch assuming dominance, but the replacement leader has also been severely damaged.

Parrot damage was first observed in a plantation at Quindalup in 1989. A subsequent survey found that damage was widespread, albeit patchy in distribution (Massam. 1992). In 1993, a grid survey of a 75 ha plantation of four year-old *E. globulus, E. saligna* and *E. botryoides* in the Wellington catchment east of Collie (700mm mean annual rainfall) found that parrot damage had caused 70 % of *E. globulus*, 62 % of *E. saligna* and 43 % of *E. botryoides* to develop a double or multi-stemmed form. In all, 98 % of trees were damaged, and it was estimated that only one third of the plantation would be worth growing on to harvest at a much reduced yield (Beatty and Ritson, 1993). This plantation was harvested in 1997 but yields and the costs of harvesting are unavailable (P.Beatty, Department of Conservation and Land Management, pers. comm.)

Eleven other one to four year-old *E. globulus* plantations were surveyed in the drier (600-700 mm rainfall) region in the same year (1993). Damage by parrots was highly variable between and within plantations (Ritson, 1993). At one plantation, only 4 % of the two year-old trees were damaged, even though parrots were common in and around the plantation, while at six plantations more than 50 % trees were damaged. There was no evidence of any pattern to the distribution of the damage in the plantations surveyed.

Further surveys suggested that plantations in the high rainfall region were usually least affected, with the exception of those near Margaret River, on the western edge of the jarrah (*E. marginata*) forest (R. Quaife, Bunnings Treefarms, pers. comm.). The additional surveys also suggested that there was a trend for greater levels of commercial damage among more recently established plantations. These was no commercial damage in plantations established prior to 1989, but 42 % of plantations established between 1989 and 1991, and 62 % of plantations established between 1992 and 1994 were damaged. This trend of increasing damage is a major concern to the industry.

A number of possible reasons for the apparent increase in parrot damage have been suggested by Ritson (1995). These include:
(i) Other sources of food have declined, forcing the parrots to explore new food sources,
(ii) Parrot populations have increased resulting in an increased demand for food sources,
(iii) Bark stripping by parrots is a 'learned' behaviour requiring some time for the technique to be passed on.

Clearly, (i) and (ii) are suggesting that parrots damage trees to obtain a food source. The link between tree damage and food availability is a credible one but there have been no studies quantifying the availability of food resources for this species in south-western Australia to ascertain whether food is in short supply. Equally, there have been no studies to determine the potential food value of the eucalypt stems.

1.2.1 Food resources for *B. zonarius*

Parrots are generalist and opportunistic feeders using a wide variety of seeds of native and introduced crop, pasture and weed species, as well as insect larvae, lerp, berries, nectar, flowers, buds, corms, immature marri (*Corymbia calophylla*) fruit and cultivated fruits (Forshaw, 1969; Long, 1984; Crome and Shields, 1992). In the agricultural areas, where most of the landscape has been cleared of native vegetation, parrots have a high dependence on paddock feed (Saunders *et al.*, 1985; Lynch and Saunders, 1991).

The importance of cultivated grain (oats and wheat) in the diet of *B. zonarius* is well established. Long (1984) found that oat grain was present in the crop of *B. zonarius* in all seasons but was particularly important in the winter diet. Oats are fed to sheep during autumn and winter when pasture quality is poor, and parrots flock to silos and feed on spilt grain. Ritson (1995) observed an increase in plantation damage in winter when spreading of oats to feed sheep in a nearby paddock ceased, and when an open bin used for storing oats was emptied. He also observed that damage decreased during late summer to early autumn

(February to March) when there was a heavy nectar flow in nearby flowering marri (*C. calophylla*). Similarly, a heavy flowering of marri dramatically reduced the number of parrots, and the amount of damage caused by parrots in orchards in the south-west (Halse, 1986). The use of nectar by *B. zonarius* is recognised (Forshaw, 1969; Long, 1984; Halse, 1986) but its importance in their diet has not been quantified.

Parrots inflict most damage in plantations in summer and autumn (December to May) (Ritson, 1995), a time when parrots are known to also feed in orchards (Long, 1985; Halse, 1986) and on grass-trees (*Xanthorrhoea preissii*) (McNee, 1997). This suggests that parrots seek to gain some food value from tree stems in response to food shortages in summer and autumn. It also suggests that oat grain and nectar may be sought in preference to eucalypt stems, and that spatial differences in food availability also contribute to differences in damage found between plantations. (Copies of data from unpublished literature can be provided if required).

Psittacines generally have a great diversity of food habits in the wild (Cannon, 1981; Jones, 1987; Rowley, 1990; Ullrey *et al.*, 1991). While primarily seed-eaters, they regularly supplement their diets with small quantities of other items such as blossoms, fruit, insects and green vegetable matter. This diversity, often determined by availability of foods, presumably allows nutritional needs to be met and minimises the risk of nutrient deficiency. However, a heavy reliance by *B. zonarius* on cereal grain in agricultural areas may predispose them to nutritional imbalances. De-hulled oat grain is reported to contain 18.2 % protein and 0.61 % lysine, the most common limiting amino acid. These values are below those recommended for similar-sized birds by the National Research Council (NRC, 1984) e.g. 24 % protein and 1.4 % lysine for Japanese quail. Other high protein food may therefore be required to balance their diet.

1.2.2 Changes in the population status of *B. zonarius*

Evidence suggests that the number of *B. zonarius* may have increased in the early part of this century, and that there was a marked increase by the early 1950s (Saunders and Ingram, 1991). It is now the most abundant parrot, and possibly the most common bird in the wheat-belt (300 - 600 mm rainfall) of Western Australia (Lynch and Saunders, 1991; Saunders and Ingram, 1991). A recent mail survey of the agricultural area of Western Australia suggests that population of *B. zonarius* increased in 14 shires from Beverley to Tambellup between 1970 and 1990 (Mawson and Long, 1995). This area coincides with the zone of most severe parrot damage (Ritson, 1995) suggesting a density-dependent relationship between parrot population numbers and damage to plantations. In addition, the seasonal increase in damage during summer and autumn (Ritson, 1995) coincides with large numbers of parrot following the breeding season (Long, 1985). Halse (1986) argued, however, that periodic 'outbreaks' of damage to orchards could be explained by a larger proportion of the normal parrot population moving out of forests into orchards due to food shortages, without the population increasing significantly.

No reliable estimates of parrot abundance are available to verify a density-dependent relationship, either on a regional or plantation basis. Fixed-width strip transects have been used successfully for determining the occurrence and density of birds (including *B. zonarius*) in the Kellerberrin district (rainfall 336 mm) (Lynch and Saunders, 1991), but few counts have been made in the higher rainfall region of the south-west. Estimates of population density of *B. zonarius* in forested areas vary from 0.12 ha^{-1} in jarrah forest near Serpentine to 0.74 ha^{-1} in karri forest near Manjimup (Halse, 1986).

Populations of *B. zonarius* have seemingly increased in areas where many other bird species, including other parrot, cockatoo and lorikeet species, are in decline because of overclearing of agricultural land and loss of habitat (Saunders, 1977; Lynch and Saunders, 1991; Saunders and de Rebeira, 1991; Mawson and Long, 1995; Mawson and Johnstone, 1997). Other parrot species to have benefited from agricultural development in Australia include the galah (*Cacatua roseicapilla*), little corella (*C. sanguinea*), long-billed corella (*C. tenuirostris*), cockatiel (*Nymphicus hollandicus*), sulphur-crested cockatoo (*Cacatua galerita*) and some of the rosellas (*Platycercus* spp.) (Ford, 1989). The galah in particular has expanded dramatically with the clearing of woodlands and forests and with the provision of grain and reliable water supplies on farms (Saunders *et al.*, 1985).

1.2.3 Bark stripping of *E. globulus* as a 'learned' behaviour

The increase in damage to *E. globulus* since 1989 may reflect a learned behaviour as *B. zonarius* adapt to a new food source. Similar changes in behaviour have been observed in redpolls (*Acanthis flammea*) in New Zealand that eat fruitbuds (Wiens and Johnston, 1977). Variation in tree damage among plantations despite generally large populations of parrots may also support this view. However, many trees in native forests and in remnant vegetation have identical damage on stems and branches (pers. observ.), which suggests that parrots may have had the ability to access this food resource for some time.

1.3 Breeding Biology in *B. zonarius*

Breeding is particularly successful in this species (Forshaw, 1969; Long, 1990; Crome and Shields, 1992) and may account for its abundance. The central and southern populations of *B. zonarius* breed between August and February, and if conditions are favourable, two broods may be reared, while birds from the northern populations are more opportunistic and may breed at any time following good rains (Forshaw, 1969). *Barnardius zonarius* show interest in nesting hollows in south-western Australia as early as February (Long, 1990). Females enter nesting hollows around June, and lay four to seven eggs from August to early October. Young fledglings leave the nest from October to early December so that the total number of days a nest was occupied, from the first laying of eggs till the last young fledged, ranged from 88 - 105 days. *Barnardius zonarius* had a 75 % breeding success (percentage of eggs which yielded fledglings), greater than the other three species of parrots studied by Long: red-capped parrot (*Purpureicephalus spurius*) (27 %), regent parrot (*Polytelis anthopeplus*) (64 %), and western rosella (*Platycercus icterotis*) (72 %). Nestling success (percentage of nestlings that fledged) was 92 %, also well in excess of the other species studied. The breeding success of *B. zonarius* is considerably greater than for many other Australian birds (15 - 58 %, Ford 1989).

Availability of nesting hollows was not limiting to parrot populations, according to Long (1990). Only 31 % of suitable hollows were used by *B. zonarius*, and many of the same nests were used each year. Saunders (1979) argued similarly for white-tailed black cockatoos (*Calyptorhynchus baudinii latirostris*). The number of suitable hollows in these studies, based mainly on hollow dimensions, may have been overestimated as factors other than hollow dimensions may denote suitability. Susceptibility of hollows to flooding, for example, is an important consideration (Rowley, 1990). Social aspects are also important in the selection of nesting hollows whereby a preference is shown for those hollows in close proximity to another nest. For the galah (*Cacatua roseicapilla*), 85 % of nests were within 10 - 80 m of another nest (Rowley 1990), while for *B. zonarius* the distance between nests varied from 17 - 52 m (Long, 1990).

Despite the apparent abundance of nesting hollows, breeding pairs of *B. zonarius* and other parrot species (eg red-capped parrot, galah) compete for suitable sites (Rowley, 1990; Long, 1990). Nesting hollows in the branches of live or dead eucalypts are selected and defended over many months prior to egg laying. Tail wagging and chattering are characteristic behaviours over this period. Breeding pair bonds are apparently permanent (Crome and Shields, 1992), with pairs remaining close to their breeding sites throughout the year (Long, 1990).

Ford (1989) suggested that predators, brood parasites, extreme weather and unexpected food shortages were the most significant causes of failure in rearing nestlings of birds. Psittacines are altricial birds whereby the young are hatched blind and are unable to feed themselves or to thermoregulate. Thus the nestlings are particularly prone to extremes of temperature and localised food shortages. The impact of predators on *B. zonarius* is unknown. Feral cats are an important predator of birds in central Australia (Paltridge *et al.*, 1997), particularly of ground-feeding birds and those that drink frequently at waterholes. Raptores are presumably also important in a natural setting, but there is little information available on this subject. Dyer and Ward (1977), however, considered there to be ample evidence that predators do not regulate granivorous bird populations, and this area of research is not further assessed.

1.4 Environmental Physiology of *B. zonarius*

Parrots, except lorikeets and a few other species, are primarily seed eaters, and are dependent on suitable sources of drinking water to offset the low water content of seeds (8 – 12 % by weight) (Fisher *et al.*, 1972). Fisher *et al.* (1972) related the drinking habits of desert birds to their diet. Of the 22 species of birds classed as granivores, all but one were water-dependent while only 17 % of insect-eaters proved to be water-dependent. These authors suggested that while *B. zonarius* was primarily a seed-eater, it also had capacity to feed on flower buds and green fruits of eucalypts (and insects) when these were available. Such changes in food habits were likely to exert a primary influence on drinking habits.

Fisher *et al.* (1972) found that *B. zonarius*, and several other platycercine parrots, confined their drinking to two relatively short periods during the day, one in early morning and the other in late afternoon. No drinking activity was observed during the middle of the day, possibly in response to high daytime temperatures and intense solar radiation. Activity can increase the body temperature of birds by 2 - 3° C, and at ambient temperatures exceeding 40° C, this increase can be critical (Dawson and Bartholomew, 1968).

Nicholls (1980) compared the physiology of *B. z. zonarius* to *B. z. semitorquatus* to examine how the *Barnadius* genus might generally have adapted to arid environments. In laboratory studies, *B. z. zonarius* had a lower energy requirement, lower food intake, and a markedly lower basal metabolic rate than *B. z. semitorquatus*. In addition, *B. z. zonarius* consumed less water at 25° C and showed better tolerance to dehydration. Neither could maintain body weight without water. *Barnardius z. zonarius* had greater heat conductance in summer and less heat conductance in winter than *B. z. semitorquatus*, so were able to withstand a wider range of temperatures. This characteristic was attributed to the greater yellow coloration of ventral feathers in *B. z. zonarius*, compared with *B. z. semitorquatus*, which was shown to reflect heat more efficiently.

A further point of interest in the earlier mentioned study of Fisher *et al.* (1972) concerns the aversion of birds, including *B. zonarius*, to drinking water from a water hole in the Murchison River, which had received less than 75 mm rain in the past seven months. The total concentration of major cations was slightly greater than that of plasma and slightly salty to taste. Sodium concentrations were greater (approximately 0.17 M) than in other water sources used by the birds. The ability to tolerate saline water, particularly for granivorous birds living in semiarid environments, is critical to their survival. Most landbirds cannot maintain bodyweight on solutions stronger than 0.3M NaCl (or 50 % seawater) although Lindgren (1973) demonstrated that the rock parrot (*Neophema petrophila*), from waterless islands off the coast of Western Australia, could tolerate 75 % seawater.

For granivorous birds, supplementation of their diet with succulent food may offer an effective way of obtaining tissue-bound water when surface water is scarce or is too saline, or when access to the surface water might predispose the birds to an unacceptably high risk of predation. Succulent insect and plant food material may be the only source of water for nestlings of the majority of bird species (Dawson and Bartholomew, 1968). Food that is rich in water content and poor in sodium would benefit parrots, which could theoretically survive without drinking on a food source with 68 % water content (Nicholls, 1980). The drinking patterns of *B. zonarius* and its tolerance of saline water in the south-west of Western Australia have yet to be critically examined.

Salinity has increased in many rivers and creeks in the south-west of Western Australia as a result of clearing native vegetation for agriculture (Olsen and Skitmore, 1991). Increased salinisation of agricultural land has given impetus to large-scale tree planting operations in many areas in an effort to lower the water table. It is possible that the increased level of damage to the stems of *E. globulus* in late summer and autumn is related in part to the declining availability of fresh water at this time of year.

1.5 Damage to trees by other birds and other animals

1.5.1 Damage to trees by birds

A variety of birds, including many parrot species, damage the stems and tops of trees in a similar fashion to that described here for *B. zonarius* in plantations of *E. globulus* (Ritson, 1995). Many are in search of food but other reasons for bark stripping include beak cleaning, nest lining, and perching.

A few species are known to feed on the sap and cambium of trees. For example, a New Zealand parrot, the south island kaka (*Nestor meridionalis*), is a nectivore that feeds on phloem sap exudates of southern rata (*Metrosideros umbellata*) (O'Donnell and Dilks, 1989). This parrot used two methods of sap feeding. The first involved stripping the bark from a branch and licking the sap exuding from the exposed surface of the cambium. The second, more specialised method, involved using its lower mandible to cut a trapdoor in the bark and then gouging a series of tiny holes into the cambium 6-10 mm below the surface. The trapdoors left distinctive scars on the trunks and damage was so severe that hundreds of scars were visible on a tree. Kaka frequently revisited some trees to lick the sap leaking from wounds, while other surrounding trees were not visited at all. Parrots fed on sap mainly in late winter and spring, when no flower or nectar sources were available. This sap feeding was regarded (O'Donnell and Dilks, 1989) as a means of providing a high energy food during times of seasonal shortage, and when energy demands were greater (winter). One might then regard the kaka as providing an important link between nectar feeding and phloem sap feeding among parrot species, and a precedent for the present study.

The sugars in nectar and phloem sap require little intestinal processing, and are rapidly absorbed and metabolised. Extraction efficiency in rainbow lorikeets (*Trichoglossus haematodus*) of glucose was 98% while that for sucrose was 90.5 % (Karasov and Cork, 1996). Clearly nectar and phloem sap are important food sources for parrots. Some species depend on it almost entirely for their energy requirements, while others use it more opportunistically when preferred resources are in short supply (Franklin, 1999).

In the northern hemisphere, sapsuckers (*Sphyrapicus* spp.) and other woodpeckers feed on the sap and cambium of a range of deciduous trees (Kilham, 1964; Oliver, 1968; Hildahl, 1978). The best known example is the yellow-bellied sapsucker (*Sphyrapicus varius*) which 'drills' holes into the trunks of trees in distinctive patterns to feed on the cambium and phloem sap. Large populations of sapsuckers can severely wound and kill forest trees, mainly as a result of wood rot and decays subsequent to the damage. They develop and maintain 'sap orchards' by repeatedly tapping favourite trees to keep the sap flowing throughout summer and autumn. Their choice of trees is unaffected by bark thickness, tree diameter, tree vigour or dominance (Oliver, 1968).

Various cockatoo (*Cacatua*) species remove bark from eucalypts to feed on insects (Brown, 1968; Tanton, 1968); for example, Major Mitchell's cockatoo (*C. leadbeateri*) chew on branches to extract larvae (Rowley *et al.*, 1989). Chewing by sulphur-crested cockatoos may be responsible for killing the growing tips of hoop pine (*Araucaria cunninghamii*) grown in plantations in south-east Queensland (Bomford, 1992). Galahs chew the bark on the trunk of a range of smooth-barked eucalypts exposing the cambium. The edges of the resultant scars are chewed repeatedly, thus increasing size of the scars. The scar itself is used for bill stropping. Some scars can be sufficiently large to ringbark and kill the tree. Galahs also cut sprays of leaves to line their nests (Rowley, 1990).

A variety of birds feed on apical buds and shoots of pines e.g. blackgame (*Lyrurus tetrix*) (Palmar, 1968) and capercaille (*Tetrao urogullus*) (Andersson *et al.*, 1970) feed on scots pine (*Pinus sylvestris*). Blue grouse (*Dendragrapus obscurus*) feed on ponderosa pine (*P. ponderosa*) (Curtis and Elder, 1965) while grosbeak (*Pinicola enucleator*) feed on white pine (*P. stobus*) (Stark, 1964). Parrots and cockatoos cause extensive damage to fruit and nut trees (e.g.

Forshaw and Cooper, 1981; Bomford, 1992), but these will not be considered further in this review.

1.5.2 Damage to trees by arboreal vertebrates

Tree trunks and upper stems are damaged by several species of arboreal mammals seeking phloem sap. In eastern Australia, sugar glider (*Petaurus breviceps*) and yellow-bellied glider (*P. australis*) cut V-shaped notches into the bark of eucalypts to the depth of the cambium layer and feed on sap exudates (Smith, 1982; Smith and Russell, 1982; Henry and Craig, 1984; Goldingay, 1987; Kavanagh, 1987; Mackowski, 1988). Mountain brushtail possums (*Trichosurus caninus*) and common brush-tailed possums (*T. vulpecula*) strip bark and scrape the cambium from a variety of Australian pines (McNally, 1955; Barnett *et al.*, 1977), while common brush-tailed possums chew on upper stems and defoliate eucalypts in northern Tasmania (C. McArthur, pers, comm.). Bark-gouging to produce sap flow has also been reported for a few species of marmosets (*Cebulla pygmaea* and *Callithrix* spp.) in South America (Coimbre-Filho and Mittermeier, 1976).

Damage to trees by the sap-feeding gliders in native Australian forests is more sporadic than was found for mountain possums in pine plantations. Less than 1% of suitable trees were incised for sap by yellow-bellied gliders (Goldingay, 1987). Some trees were used repeatedly, and were actively defended from other animals at certain times of the year. Generally, only a few eucalypt species are used for sap feeding: *E. resinifera* in north Queensland (Smith and Russell, 1982) and *E. viminalis* in New South Wales (Goldingay, 1987). In a study in Victoria, only three (*E. globulus, E. bridgesiana* and *E. cypellocarpa*) out of eleven species of eucalypts were used for sap feeding, and then only some trees were selected (Henry and Craig, 1984), which may have been related to their ability to produce a consistent sap flow. Goldingay (1987, 1991) found that sap flow was significantly greater in trees 'in use' than in those 'not in use', but found no relationship between soil moisture or rainfall and sap flow. Sap flow was extremely variable among trees of a given species, with individual trees exhibiting pronounced variability throughout the year.

Kavanagh (1987) noted that the use of particular eucalypt species by yellow-bellied gliders was related to the fertility of the site occupied by the species at different altitudes. At his study site (800 m altitude), only two eucalypt species, *E. viminalis* and *E. fastigata*, were used for sap collection, while *E. obliqua* and *E. cypellocarpa*, which were confined to ridges, were ignored. In nearby coastal forest, where *E. obliqua* and *E. cypellocarpa* grew on more fertile and productive sites (gullies and creek flats), these species were used extensively.

Gliders eat insects, phloem sap, nectar, manna, honeydew, lerp, pollen and other high energy food, with the amount of each apparently interchangeable, depending on availability (Smith, 1982; Henry and Craig, 1984; Kavanagh, 1987; Goldingay, 1991). Sugar gliders and yellow-bellied gliders fed on phloem sap and nectar mainly in autumn and winter in temperate eastern Australia, when insect abundance tended to be low (Smith, 1982; Henry and Craig, 1984). In summer, insects were the preferred food source, even though sap was still available. In northern Queensland, however, where sap production was more continuous, yellow-bellied gliders fed on phloem sap all year round (Smith and Russell, 1982). In that environment, nectar was apparently only consumed in spring despite trees flowering for much of the year. A relatively low volume of insects was recorded in the diet of gliders, which suggested their protein intake may have been restricted. Kavanagh (1987) found the pattern of foraging by yellow-bellied gliders depended on the particular species of eucalypts present and on their phenology (flowering, new shoot growth and bark shedding), and that a mosaic of tree species was required to provide continuity of food resources.

There is a striking similarity between mountain possum damage in pine plantations in New South Wales and *B. zonarius* damage in *E. globulus* plantations in Western Australia. Possums strip the bark from the lead shoot of pines and scrape the cambium for food. The bark is not eaten and is dropped to the ground. Possums often return to damaged trees and feed on the callused edges of old wounds. Severe damage may kill trees. Strips of phloem tissue rich in starch were found among the stomach contents of these animals (McNally, 1955). Barnett *et al.* (1977) surveyed tree damage using a classification similar to that used by Ritson (1995) for *E. globulus* plantations. Damage was greater where pine plantations adjoined wet sclerophyll and rain forest, (the primary habitat of mountain possum), than when plantations adjoined dry sclerophyll forest.

Species of marmoset (*Callithrix* spp.and *Cebulla pygmaea*) gouge holes in fresh branches of several species of trees in Brazil by scraping the bark with their lower anterior teeth. Exudates are either licked or collected with the teeth. The gum is a high energy food containing 84 % (dry weight basis) carbohydrate and significant concentrations of several minerals (Coimbre-Filho and Mittermeier, 1976).

These findings, together with the previously mentioned studies of nectarivorous parrots and woodpeckers, suggest that *B. zonarius,* like these other groups of animals, may access phloem sap and cambium from *E. globulus* and other eucalypts deliberately as a valuable alternative food, or nutritional supplement, when either nectar or other high energy food is in short supply.

1.5.3 Damage to trees by terrestrial animals

A large number of terrestrial animals, both wild and domestic, browse on woody stems, e.g. moose, snowshoe hares, reindeer, caribou, tundra vole, ptarmigan, goats and sheep (in Bryant and Raffa, 1995). In Australia, wallabies, rats and quokka damage the stems of pine trees (McNally, 1955). Damage by terrestrial animals is generally confined to young trees up to six years old, or until the bark becomes too thick and hard, in contrast to arboreal animals which can attack trees of any age by attacking the upper stems with softer bark (McNally, 1955).

Considerable research into the browsing damage by deer in Britain and other northern temperate regions has highlighted several important factors (see review by Gill, 1992). Deer damage trees by browsing, bark stripping and by fraying. Bark stripping is usually confined to winter, although some species are only stripped in summer. Bark stripping on beech was positively correlated to rainfall, and it was suggested that increased rainfall could make the bark easier to remove. While it is assumed that bark is used as a food, no simple relationship between the nutrient content of bark and damage was found.

1.6 *Barnardius zonarius* as a bird pest species

Barnardius zonarius has been damaging fruit orchards since the early part of the 20th century and was declared vermin throughout the state as early as 1921 under the Vermin Act (Serventy and Whittell, 1967). They remain a pest in many orchards (Long, 1985; Halse, 1986), causing damage to commercial flower crops, vineyards and domestic gardens, and have killed many native grass-trees (*Xanthorrhoea preissii*) (McNee, 1997). There is an open season for shooting Twenty-eight parrots in many shires in the south-west. Shooting and (illegal) poisoning of parrots has long been practised to protect horticultural crops in this region.

Species are classed as pests when their utilisation of an ecosystem conflicts with that of man (Wiens and Johnston, 1977). A comprehensive review (Bomford, 1992) highlighted significant agricultural losses caused by birds in Australia, mainly to dryland cereal and oilseed crops, but also to irrigated rice and to fruit orchards. While losses to industries are often low, losses to individual growers can be severe. These losses are patchily distributed in space and time, and are thus unpredictable. Native psittacines are among the major bird pest species in Australia, along with corvid species, silvereyes (*Zosterops lateralis*) and European starlings (*Sturnus vulgaris*).

Granivorous birds have been among the most successful in taking advantage of agricultural development, but of the hundreds of such species in the world, only a few have become serious pests (Dyer and Ward, 1977). The ability of some granivorous species to exploit patchily distributed seed sources is related to certain adaptive features (Wiens and Johnston, 1977). These include:

- mobility and ability to locate and rapidly exploit locally concentrated food sources,

- the formation of large flocks for feeding,
- opportunistic and generalist feeding habits,
- the ability to learn locational or visual cues for productive food patches,
- well developed distensible crop to allow them to eat rapidly and store the seed,
- high reproductive output (e.g. large clutch size, multiple broods, rapid development rates, early sexual maturation),
- insensitivity to conventional control methods.

Many of these features are evident for *B. zonarius*, some of which have already been mentioned (e.g. feeding habits, reproductive output). This species seldom forms large flocks, other than where feed is locally abundant (e.g. around grain silos and orchards), and even then flock sizes are rarely greater than 100 individuals. This contrasts with some other parrot species in south-eastern Australia where feeding flocks of up to 7000 long-billed corellas (Alexander, 1990) and 5000 galahs (Bennett, 1978) have been reported.

1.7 Management and control of bird pests

Control of wildlife pests usually aims to reduce animal numbers to some previously stable state. However, when pest populations are reduced, the fecundity and survival of the remaining population often increases, unless the availability of resources is also reduced (Caughley and Sinclair, 1994). Rarely does a direct reduction in the population result in proportionately reduced damage levels. Management of pests is considered to be more cost-effective when focussed on reducing damage rather than on reducing pest numbers (Dyer and Ward, 1977; Braysher, 1993).

Bomford (1992) asserted that shooting was the most widely practised, yet the most ineffective, technique for reducing bird damage in Australia. For example, despite reducing the population of long-billed corellas by 90 %, South Australian farmers still expressed concern at the widespread damage caused by this species. Obviously, shooting was effective in controlling the population of long-billed corellas, but population reduction may not be effective where the crop being damaged is the preferred food resource. In these cases, other strategies, such as growing alternative crops, may be the only effective way of reducing damage.

Halse (1986) commented on the tendency of populations of several species of parrots to remain fairly constant around orchards despite widespread shooting. Nearly all parrots shot in orchards were juveniles, and the loss was probably compensated by a reduction in natural mortality of the remaining flock. When damage is caused by mostly non-breeding flocks, shooting seems to have a negligible effect on population (Ford, 1989). However, when breeding birds join the flock, shooting can have a devastating effect. Widespread shooting and poisoning of Baudin's cockatoos from the 1950's until early 1990's to reduce damage to apple and pear crops, combined with extensive clearing of its native habitat, and low fecundity, have resulted in the listing of this species as threatened fauna in Western Australia (Saunders *et al.*, 1985; Mawson and Johnstone, 1997).

Professional shooters employed to reduce parrot numbers around plantations of *E. globulus,* shot over 7000 parrots in the first six months of operation (R.Quaife, pers. comm.). Despite Bomford's assertions, this culling appears to have reduced damage to nearby plantations (pers. observ.). Combinations of shooting, trapping and diversionary feeding have also been tried in plantations of *E. globulus* with mixed success (Ritson, unpublished data). Other methods used in Australia include decoy cropping, screening, scaring devices, netting, chemical repellents, breeding for bird resistance, and fertility control (Long *et al.*, 1989; Fleming *et al.*, 1990; Bomford, 1992).

1.8 Tree factors influencing rates of herbivory

According to a number of recent models (Bryant *et al.*, 1983; Coley, 1983; Coley *et al.*, 1985; Coley, 1988; Price, 1991), faster growing species are considered more likely to be damaged by herbivores than slower growing species. The 'resource availability', 'plant vigour' and 'carbon/nutrient balance' hypotheses, developed mainly from studies on insect herbivory of woody plants, suggest that faster growing plants produce fewer carbon-based chemical defence compounds (tannins and lignins), as a result of a lower carbon to nutrient balance, and are thus more attractive to herbivores. Wild browsing animals have also been shown to feed preferentially on stems of rapidly growing species characteristic of productive habitats (Bryant and Raffa, 1995). These hypotheses may also apply to differences in growth rate within species (Price, 1991), so that factors affecting the growth rate of eucalypts may be relevant to the present study.

1.8.1 Influence of soil fertility on tree growth rate and herbivory

Many eucalypt forests of Australia have evolved on infertile soils with low concentrations of nitrogen and phosphorus in particular (Beadle, 1966). As a result, their foliage is generally poor in nutrients and rich in secondary compounds including tannins, simple phenols and terpenoids (Ohmart and Edwards, 1991; Landsberg and Cork, 1997). However, trees growing on better quality sites tend to have faster growth rates and greater nutrient concentrations in their foliage (Lamb, 1977; Bell and Ward, 1984; Judd *et al.*, 1996). Eucalypts grown in plantations have greater concentrations of almost all foliar elements, reflecting increased nutrient availability due to intensive silvicultural treatments, than eucalypts in native forests (Judd *et al.*, 1991, 1995).

Nutrient concentrations in foliage from 22 eucalypt communities in south-eastern New South Wales were a major determinant of the density and species diversity of arboreal marsupials, particularly for folivores, but also for sap feeders (Braithwaite *et al.*, 1983). Cork (1992) subsequently showed that greater nitrogen, phosphorus and potassium concentrations in eucalypt foliage from these communities were associated with lower total polyphenol concentration. He concluded that both polyphenols and foliar nutrients were important in distinguishing between favourable and unfavourable sites for arboreal marsupials. In arid and semiarid woodlands, however, abundance of brushtail possum was best explained by factors related to water availability than by foliar nutrient concentration (Munks *et al.*, 1996).

Insect herbivory has been positively related to nitrogen concentrations in eucalypt foliage (Fox and Macauley, 1977; Journet, 1980; Landsberg and Wylie, 1983; Ohmart *et al.*, 1985a, 1985b; Ohmart and Edwards, 1991), which, in turn, can be related to leaf age (Landsberg, 1990; Ohmart and Edwards, 1991), and availability of soil nitrogen (Landsberg *et al.*, 1990; Adams and Atkinson, 1991; Näsholm *et al.*, 1994). Landsberg and Gillieson (1995), however, found that herbivory tended to increase as climate and site productivity generally became more favourable, and that foliar nitrogen was only one of a number of indicators associated with herbivory.

The form of nitrogen available for plant uptake can influence the concentration and composition of soluble nitrogen in leaves, which may be more important than total leaf nitrogen in determining herbivore preferences. Adams and Atkinson (1991) found greater concentrations of amino acids in xylem sap and foliage of *E. regnans* growing in soils with higher soil nitrate-to-ammonium ratios in both field and glasshouse studies. Elevated concentrations of amino acids, particularly those of glutamine, were associated with increased abundance of psyllids (*Cardiospina bilobata*). However, this is not a general trend, as some species of pine are known to accumulate greater concentrations of amino acids when supplied with ammonium-N than with nitrate-N (van den Driessche, 1974).

The influence of the form of nitrogen on herbivory has received little attention and may well be relevant for plantations of *E. globulus* grown on ex-agricultural land. In agricultural soils with higher rates of nitrogen mineralisation and lower C:N ratios, nitrate is generally the major form of nitrogen (Richards, 1976), while in temperate mature forests the predominant form of nitrogen is likely to be ammonium (Keeney, 1980). Eucalypt species appear to vary in their preference for either nitrate or ammonium for growth (Moore and Keraitis, 1971), with forest species generally showing a preference for ammonium. High concentrations of nitrate can cause growth depression and toxicity symptoms in *E. globulus* seedlings in sand culture (Shedley *et al.*, 1995), but these symptoms are rarely seen in the field.

Many plants accumulate amino acids in response to different stresses (e.g. temperature, moisture, salinity, shading and mineral deficiency) that inhibit protein synthesis and growth rather than nitrogen uptake (Rabe and Lovatt, 1986; Rabe, 1990; Vance and Zaerr, 1990). These stresses may provoke outbreaks of herbivorous insects on plants (Mattson, 1980; White, 1984; Mattson and Haack, 1987). The 'plant stress' and 'plant vigour' models of herbivory both recognise that insect herbivory increases when the availability of plant nitrogen increases, either as a result of plant stress or plant vigour.

1.8.2 Effect of fertilisers on herbivory

Many examples from overseas literature indicate that fertilised trees are more prone to browsing damage than unfertilised trees (Brockley, 1988; Bryant and Raffa, 1995). Gessels and Orians (1966) found that rodents damaged 36 % of fertilised pacific silver fir (*Abies amabilis*) compared with 6 % of unfertilised trees. The tops of scots pine were damaged more frequently and severely by capercaillie when fertilised with calcium and nitrogen than those given potassium or phosphorus fertiliser (Andersson *et al.*, 1970).

In a feeding trial with common brushtail possums in eastern Australia, seedlings of *E. blakelyi* irrigated with a nutrient solution were preferred to seedlings irrigated with water only (Landsberg, 1987). The nutrient-fed seedlings contained more water, nitrogen, soluble sugar and fibre, and less tannin, starch and non-structural carbohydrates than the water-fed seedlings.

Large growth responses to nitrogen and phosphorus, particularly together, have been demonstrated for many eucalypt species in field trials (Cromer *et al.*, 1981; Cromer and Williams, 1982; Schönau and Herbert, 1982; Ward *et al.*, 1985;

Grove, 1988; Yost *et al.*, 1987; Schönau and Herbert, 1989; Cromer *et al.*, 1991; Birk and Turner, 1992; Judd *et al.*, 1996). These responses are modified by rainfall, soil depth and structure, and soil fertility status (Schönau and Herbert, 1989; Cromer *et al.*, 1991; Judd *et al.*, 1996). Growth responses are well understood by the eucalypt plantation industry, in comparison to the poorly known effects of fertilising, or of variations in site fertility, on herbivory. The fact that concentrations of nutrients in foliage generally increase following the addition of fertiliser to young eucalypt plantations on responsive sites (Cromer *et al.*, 1981; Schönau and Herbert, 1982; Yost *et al.*, 1987; Judd *et al.*, 1996), suggests that management practices are likely to affect the level of herbivory.

1.8.3 Moisture stress and herbivory

The other major determinant of growth rate in eucalypts is availability of soil water (Herbert, 1990; Tomé and Pereira, 1991; Honeysett *et al.*, 1996; Judd *et al.*, 1996; Hingston and Galbraith, 1998; Hingston *et al.*, 1998; White *et al.*, 1996). Some species shed leaves to avoid the effects of drought; for example, *E. maculata* shed 50 - 97 % of its pre-drought leaf area during a seven-month drought (Pook, 1986). *Eucalyptus marginata* has a deep rooting pattern that allows access to soil moisture at depth (Dell *et al.*, 1983a). *Eucalyptus globulus* and *E. nitens* typically respond to water stress by stomatal closure rather than by leaf shedding or adjustment of osmotic or elastic properties of their leaves (Pereira *et al.*, 1992; Osório and Pereira, 1994; White *et al.*, 1996; David *et al.*, 1997). White *et al.* (1996) concluded that these less dramatic responses to water stress in *E. globulus* allow it to survive and grow under moderate or seasonal water stress, but did not confer good adaptation to severe water stress. Pereira *et al.* (1992) showed that the reduction in net photosynthesis of three-year-old *E. globulus* during periods of water stress was due to stomatal closure rather than reduced photosynthetic capacity. Under moderate water stress, stomatal closure and reduced leaf expansion led to lower transpiration and reduced growth rates (Osório and Pereira, 1994).

Significant areas of *E. globulus* plantations on certain soil types died of water stress during prolonged dry summer conditions in the south-west of Western

Australia in 1993/94 (Dutkowski, 1995), and in 1997/98 (pers. observ.). Trees most at risk were four to five years old, at maximum leaf area, or 'canopy closure', and growing on sites where soil moisture storage was less than required for growth over the hot dry summer period. At Mumballup, six-year-old trees died from drought in May 1994 due to shallow soils (1.5 m to rock), and inadequate soil water storage capacity (250 mm), even though mean annual rainfall was 950 mm (Hingston *et al.*, 1998).

Plants often respond to drought stress by accumulating solutes in a process termed 'osmotic adjustment', allowing maintenance of cell turgor pressure. Solutes such as sugars, amino acids, organic acids and potassium accumulate in leaves, roots and inner bark of trees (Myers and Neales, 1986; Vance and Zaerr, 1990; Kozlowski and Pallardy, 1997). For example, in apple trees, the concentration of total sugars increased nearly 50 % in young, fully expanded leaves in response to drought conditions (Fanjul and Rosher, 1984), possibly as a result of increased breakdown of starch from stem and roots (Wang *et al.*, 1995).

Water stress is also known to cause changes in sucrose concentration, rate of phloem transport and rate of exudation of phloem sap (Kursanov, 1984; Kozlowski and Pallardy, 1997). Hall and Milburn (1973) demonstrated that in castor bean (*Ricinus communis*), rates of exudation of phloem sap from small incisions in the bark decreased sharply when water was withheld, while concentrations of sucrose in phloem increased. Fluctuations in exudation rates were large in comparison to changes in sap concentration. Although it has been noted that exudation rate is greater in healthier, faster growing plants (Milburn, 1971; Hocking, 1980), the physiological basis for such variation has not been satisfactorily clarified. Phloem transport is thought to be driven by osmotic pressure (Münch's hypothesis) with the rate of flow determined partly by the size of gradients in solute concentration between 'source' leaves and various 'sinks' (Milburn, 1974, 1975). Hence, factors that influence either photosynthesis in leaves, or metabolism in sinks, would be expected to modify the rate of phloem transport. Water stress appears to influence phloem sap concentration and flow rate as a result of reduced sink metabolism and osmotic adjustment. Given this scenario, it seems surprising that no relationship was found between the rate of flow of phloem sap in eucalypts, and rainfall or soil moisture in the previously mentioned studies on yellow-bellied gliders by Goldingay (1987, 1991). Possibly soil water storage capacity, or soil depth, may have been a more reliable indicator of the availability of soil water (see above).

Induced water stress in loblolly pines (*Pinus taeda*) caused a marked increase in concentrations of reducing sugars, non-reducing sugars and total carbohydrates in inner bark as a result of reduced growth, and this was related to increased susceptibility of attack by bark beetles (Hodges and Lorio, 1969). In eucalypts, water stress facilitated the invasion of long-horned wood borers (*Phoracantha semipunctata*), but this was believed to be related to physical effects, whereby young larvae were killed as they attempted to penetrate bark with a high moisture content (Powell, 1982; Paine *et al.*, 1995). Other insects are reported to have peak feeding responses to relatively high sucrose levels (0.01 - 0.05 M), as a result of water stress (Cobbinah *et al.*, 1982; Mattson and Haack, 1987). There seems a plausible hypothesis that the high level of parrot damage reported in plantations of *E. globulus* in the drier areas of Western Australia may be due to increased sucrose concentration in the phloem sap as a result of water stress.

1.8.4 Genetic variability in resistance to herbivores

In the northern hemisphere, bark stripping and browsing by deer varied markedly with tree species and provenance (Gill, 1992). Reasons for this selectivity are not known, but presence of certain morphological characters, such as low branches, short internodes, thick bark, rough bark or bark which adheres strongly, deters animals from this behaviour. Arnold and Hill (1972) and Harborne (1993) have reviewed chemical factors affecting food selectivity in animals, while Provenza (1994) reviewed behavioural and social aspects.

Species and provenances of eucalypts vary greatly in their resistance to insect herbivores (Edwards *et al.*, 1993; Farrow *et al.*, 1994; Floyd *et al.*, 1994). In *E. globulus*, the King Island provenance was more resistant to autumn gum moth (*Mnesampela privata*) and leaf blister sawfly (*Phylacteophaga froggatti*) than other provenances (Farrow *et al.*, 1994). Edwards *et al.* (1993) studied six eucalypt species and found that defoliation by Christmas beetles (*Anoplognathus* spp.) was unrelated to total terpenoid concentration of the foliage, but was strongly related to the concentration of cineole in the terpenoid mixture. Resistant trees from the six species had a mean cineole concentration of 71.8 % compared with only 11.2 % for defoliated trees. These results suggest that at least some of the variability in the level of parrot damage may be related to genetic variations in physical or chemical defence characteristics of trees.

1.9 Seasonal variation in the carbon and nitrogen composition of trees

Seasonal variations in carbohydrate and nitrogenous compounds in the inner bark, cambial region and wood of deciduous trees and conifers have received much attention, but little is known for eucalypts. Deciduous trees tend to accumulate greater carbohydrate reserves than evergreens, and are affected by annual cycles of defoliation, dormancy and budburst.

1.9.1 Variation in carbohydrate composition of the inner bark and cambium of trees

Starch is the most important carbohydrate reserve, but sugars (sucrose and oligosaccharides), alcohols, fats and oils are encountered in trees (Kozlowski and Pallardy, 1997). Both stored and currently produced carbohydrates are used for growth. In trees, carbohydrates are mainly stored in bark tissue and in ray and xylem parenchyma cells of stems and roots. Starch grains are abundant in ray cells near the cambium and decrease toward the inner sapwood. The concentration of carbohydrates in bark is often very large, but the total amount in bark is less than in wood. Pate and Jeschke (1995) suggested that among Australian trees, fire-resistant, 're-sprouter' species often possess larger deposits of starch in xylem parenchyma than fire-sensitive, 'obligate seeder' species. Starch reserves are extensively utilised during refoliation following destruction of foliage by fire. Starch may also be stored in roots of many shrubby species commonly found in Australian ecosystems (Pate *et al.*, 1990).

In olive trees (*Olea europaea*), mannitol was the most abundant carbohydrate in leaves and bark (Drossopoulos and Niavis, 1988), although starch accumulated in xylem and bark during spring and early summer. The low concentrations of starch in bark in late winter suggested a preferential mobilisation of starch from this tissue rather than from xylem for cambial activity. Mannitol, the major soluble carbohydrate, decreased in xylem and bark as starch increased, but seasonal fluctuations were less pronounced.

Similar seasonal fluctuations in sugars and starch concentrations have been recorded for deciduous trees (Catesson et al., 1995; Krabel *et al.*, 1993). Red osier dogwood (*Cornus sericea*), a cold-tolerant species, maintained greatest concentrations of soluble sugars, in both bark and wood tissues, in midwinter when concentrations were three times greater than in summer (Ashworth *et al.*, 1993). In early spring, concentrations of soluble sugars decreased significantly, and the concentration of starch increased to peak at bud break. Sugar composition also varied seasonally. Raffinose in particular showed dramatic changes in concentration, and appeared to parallel seasonal changes in cold-hardiness. In winter, raffinose increased to one fifth of the total sugars in bark, and to one third of that in wood, but was barely detectable in summer and early autumn. Raffinose, a trisaccharide, may be more effective than sucrose as a cryo-protectant, due to its ability to vitrify and prevent further dehydration.

Composition of phloem and cambial saps from *E. regnans* reflected the seasonal growth characteristics of trees in south-eastern Australia (summer rainfall pattern) and was significantly affected by rainfall and temperature (Stewart *et al.*, 1973). Sugar concentrations in sap decreased markedly in late spring during a period of rapid growth of new leaves in the crown, and 'springwood' cells in the cambium, but increased again in summer as leaves matured. Cessation of cambial growth in winter reduced the demand for sucrose that consequently increased in concentration in the cambial region. Information on starch in eucalypts is scanty, but values found in root wood of *E. globulus* subsp. *bicostata* were 4.0 % starch and 2.5 % soluble sugar (Tomkins *et al.*, 1989). In contrast, deciduous stems may contain up to 10.0 % starch (Ashworth *et al.*, 1993).

Cambial activity is more continuous in evergreens than in deciduous trees, but remains responsive to environmental stress (Kozlowski and Pallardy, 1997). New techniques for studying environmental effects on cambial activity in eucalypts are being developed using point dendrometers, and electrodes to measure cambial hydration electrochemically (Downes *et al.*, 1999). Early results suggest that irrigation extended the period of cambial activity, and produced smooth patterns of growth, indicative of steady rates of cell division and expansion. Conversely, under rain-fed conditions, the pattern of cambial growth was irregular. Drought had a significant impact on cambial activity at the cellular level, reducing the width of the zone of cell division and enlargement, increasing wood density and causing stem shrinkage. Cambial activity is therefore expected to vary with season and site quality, and may be associated with differences in tree attractiveness and parrot damage in *E. globulus* plantations.

1.9.2 Variation in nitrogen compounds in the inner bark and cambium of trees

Young leaves and actively growing meristematic tissues such as cambia, and shoot and root tips, generally have greater concentrations of nitrogen (3 - 7 %) than other tissues. This nitrogen is required to support protein synthesis when cells are rapidly expanding in number and size (Mattson, 1980). Concentrations of nitrogen then decline until tissues senescence, when proteins are hydrolysed and amino acids are translocated via phloem to younger tissues, or stored in stem and bark parenchyma during winter, ready to support new spring growth (Kozlowski and Pallardy, 1997).

Tromp and Ovaa (1971) showed that protein in apple bark contributed a major portion of the nitrogen stored in winter that was exported to new growth regions in spring. Millard and Neilsen (1989) later showed that the major form of nitrogen remobilised and translocated during growth in apple trees were proteins rich in asparagine and arginine (low C:N). During leaf senescence in apple trees, over 80 % of retranslocated soluble protein nitrogen appears to come from degradation of ribulose 1,5-*bis*phosphate carboxylase/oxygenase (Rubisco) (Millard and Thomson, 1989).

Specific bark storage proteins have since been identified in a variety of deciduous trees (Wetzel *et al.*, 1989; Stepien and Martin, 1992; Stepien *et al.*, 1994). Vegetative storage proteins (VSPs) accumulate in protein bodies within storage vacuoles of stem parenchyma cells in inner bark (secondary phloem) and wood (xylem ray parenchyma). The concentrations of these proteins increase during late summer and autumn and remain high in inner bark throughout winter (Wetzel *et al.*, 1989). During spring, prior to and during budburst, the protein bodies are replaced by large central vacuoles as a result of mobilisation of the storage protein. Bark VSPs in poplar (*Populus x euramericana*) contain 10 - 22 % covalently bonded oligosaccharides (glycoproteins), which may also act as a carbon store (Stepien and Martin, 1992).

By contrast, in sitka spruce (*Picea sitchensis*), nitrogen was stored over winter in roots and in current-year needles (Millard and Proe, 1992). The amount of nitrogen stored was dependent on the quantity of nitrogen supplied to the trees, with a greater proportion of nitrogen stored in roots in low nitrogen treatments. Seasonal changes in amino acid composition in buds, shoot apices and leaves of white spruce (*Picea glauca*) saplings were shown to be related to the storage and mobilization of nitrogenous compounds during onset of dormancy or growth of shoots (Durzan, 1968). Metabolism of storage arginine in buds in early spring lead to an increase in glutamine, glutamic acid and proline. Arginine concentrations increased when shoot elongation ceased in summer. Arginine concentrations also increased in needles of water stressed ponderosa pine, and it was suggested that storage of ammonia, released as a result of proteolysis, was important when stress reduced carbon fixation (Vance and Zaerr, 1990).

Little is known about the transport and storage compounds of nitrogen in eucalypts, let alone their seasonal distribution patterns. Glutamine was the main amino acid found in xylem sap of *E. regnans*, while glutamate, aspartate and γ-aminobutyric acid were dominant in foliage (Adams and Atkinson, 1991). In *E. blakelyi*, proline, arginine, alanine, glutamine, phenylalanine and methionine were the dominant amino acids in foliage (Journet and Cochrane, 1978). Adams *et al.* (1995) found an accumulation of arginine in older leaves of *E. globulus*, *E. regnans* and *E. nitens* grown with abundant nitrogen, but limiting phosphorus.

Stewart *et al.* (1953) analysed the cambial region of young *E. regnans*, and found protein concentration in the cambial zone to be 12.5 %, declining to 2.7 % in the outer phloem, and to 2.8 % in developing sapwood, 0.6 % in sapwood and 0.3 % in heartwood. These figures suggest withdrawal of nitrogen during the transition from sapwood to heartwood. O'Connell *et al.* (1978) found significant withdrawal of nitrogen (and phosphorous) from leaves of *E. marginata* prior to senescence, and a marked seasonal peak in litterfall in summer.

Total nitrogen concentrations in the youngest fully expanded leaves of *E. globulus* grown on ex-forest soil in south-western Australia increased from 1.7 % in February (summer) to 2.5 % in April, following 80 mm rain (Shedley *et al.*, 1995). Corresponding increases on ex-agricultural soil were from 2.2 % to 2.5 % (Shedley, 1995), suggesting major changes in the nitrogen status of *E. globulus* over this period.

1.10 Conclusions

This project involves the interaction between a native parrot species that has reached pest status, and an exotic eucalypt species being planted as a monoculture in an environment favouring rapid growth rates. The review has presented aspects of both parrot biology and eucalypt physiology that may be relevant to the study of parrot damage in plantations of *E. globulus* in the south-west of Western Australia. The parrot, *Barnardius zonarius*, is seemingly well adapted to a changing agricultural ecosystem, with a well developed ability to locate and exploit a wide variety of foodstuffs, and a high recruitment rate. Parrot populations have increased in areas where seasonal excesses of cereal grain ensure a high rate of nestling survival, and it is in these areas that damage to plantation eucalypts has been most severe.

The suggestion that parrots are damaging tree stems to derive some nutritional benefit is supported by examples of similar types of herbivory from the literature, and from an understanding of the nutritional requirements of this parrot species. The implication from these examples, and from anecdotal evidence, is that parrots may be seeking the phloem sap and cambium of eucalypts when nectar and other energy-rich foods are in short supply, particularly in autumn and early winter.

It is apparent from earlier studies that damage varies seasonally, as well as within and between plantations, although no pattern in the distribution of damage has emerged. These seasonal and spatial variations could be due to parrot-related factors, such as differences in population dynamics, nutritional requirements, or quantity or quality of food available to parrots. On the other hand, variations may be due to tree-related factors, such as differences in accessibility of plantation trees from habitat trees, or physical and chemical attributes of trees that may determine their attractiveness and palatability to parrots. These dynamic relationships are likely to be complex and subject to many environmental influences.

Aspects of breeding biology, physiology, and diet of *B. zonarius* have been studied, but little is known about population density and dynamics, specific nutritional requirements and food availability. The growth and nutrition of eucalypts, and *E. globulus* in particular, have also received much attention, yet our knowledge of factors affecting cambial activity and phloem sap composition is poor. In contrast to deciduous trees, little is known about seasonal changes in the composition of carbohydrates and nitrogenous compounds in the inner bark of eucalypts.

Managing parrot abundance and activity by manipulating food supplies or availability of nesting hollows (the two resources most likely to limit parrot populations), will be almost impossible. Direct killing is an option currently being used, but is expensive and the long-term consequences are unknown. Diversionary feeding has been tried with limited success, while the use of bird repellents and scaring devices are unlikely to be economically feasible, given the large areas of *E. globulus* planted each year. One alternative is to understand why some trees and some plantations are attractive to parrots, and develop management options to reduce this attractiveness. The relationship between soil fertility and water stress and parrot damage is of particular interest, because these are aspects of plantation management that can be manipulated. An understanding of site characteristics which predispose plantations to parrot damage could assist in the development of a model to predict areas at high risk of damage which may improve the efficiency of current control programs. In the longer term, genetic selection of less attractive trees may be worth consideration.

1.11 Research objectives

The approach taken here is, of necessity, exploratory since little is known on the subject of parrot damage. Many aspects of the animal-plant interaction require definition before an experimental approach can be considered. Measurement and manipulation of parrot population dynamics and availability of food supplies is considered beyond the scope of this project. Instead, major emphasis is placed on assessing and understanding the patterns of damage, both in time and space, and attempting to relate these patterns firstly, to variations in site characteristics, secondly, to variations in tree nutrition and phloem sap composition, and thirdly to variation in nutritional requirements of parrots. A degree of control over some variables was afforded by the opportunity to use even-aged plantations, grown under similar establishment conditions by one company. Some aspects of diet selectivity were explored using recently caged parrots. Research objectives were therefore four-fold:

1. to determine if patterns exist in the distribution of parrot damage in plantations of *E. globulus*; and if so,

2. to identify site characteristics which best explain the distribution and intensity of damage in plantations, and develop a model to predict plantations at high risk of damage;

3. to examine the seasonal and spatial variability in phloem sap composition and sap yield in *E. globulus,* and determine the relationship of these variables to parrot damage; and

4. to test the ability of parrots to discriminate between 'attractive' and 'non-attractive' trees.

This research project should also be of value to the study of other phloem feeding animals, and in the understanding of carbon and nitrogen fluctuations in eucalypt stems.

CHAPTER 2

ASSESSMENT OF TREE DAMAGE IN PLANTATIONS OF *EUCALYPTUS GLOBULUS*

2.1 Introduction

Damage to *Eucalyptus globulus* from parrots was first observed in 1989 in plantations in the south-west of Western Australia. Since then, a number of plantations have been surveyed to determine the extent of damage (Massam, 1992; Beatty and Ritson, 1993; Ritson, 1993). These surveys have highlighted the variability in damage both within and between plantations. Damage appears to be increasing, despite efforts to control the problem. Perceived increases in the parrot population in these mainly cereal growing areas (Mawson and Long, 1995) is one explanation for increasing damage. A review of damage and possible solutions is given by Ritson (1995).

Twenty-eight parrots (*Barnardius zonarius*) damage the lead shoot of trees by stripping bark from young stems, which often results in broken tree tops and deformed stems, as described in Chapter 1. There is little understanding of the reasons behind this damage, other than the possibility that the parrots may be feeding on the cambium and/or phloem sap of the stem to obtain additional carbohydrates. Carbohydrates such as nectar, manna, lerp, honeydew and sap are important food resources for many birds (e.g. honeyeaters, silvereyes and lorikeets) inhabiting Australian eucalypt forests and woodlands, although the extent of dependence differs between bird species (Paton, 1980; Recher *et al.*, 1985; Ford, 1989; Franklin and Noske, 1999).

There are several analogous examples of animals feeding on phloem and cambium of trees, such as yellow-bellied sapsuckers (*Sphyrapicus varius*) in California (Oliver, 1968), south island kaka (*Nestor meridionalis*) in New Zealand (O'Donnell and Dilks, 1989) and a few species of marmoset (*Cebulla pygmaea* and *Callithrix* spp.) in Brazil (Coimbre-Filho and Mittermeier, 1976). Some Australian arboreal marsupials, such as sugar gliders (*Petaurus breviceps*) and yellow-bellied gliders (*P. australis*) (Smith, 1982; Smith and Russell, 1982; Henry and Craig, 1984; Goldingay, 1987) appear to feed on phloem sap of eucalypts as a substitute for nectar in times of low nectar supply. This substitution could also apply to *B. zonarius*, which is known to feed on eucalypt nectar (Forshaw, 1969; Long, 1984).

Earlier surveys of tree damage in plantations of *E. globulus* found no consistent pattern to the damage. 'Edge' trees were no more damaged than 'non-edge' trees (Ritson, 1995). Similarly, sapsucker damage to young ponderosa pine (*Pinus ponderosa*) was dispersed throughout the stand and was unrelated to bark thickness, breast height diameter, vigour, or tree dominance (Oliver, 1968). Sapsuckers appeared to be attracted to those trees that had suffered previous damage, ignoring other trees in close proximity. Damage to trees in native forests by sap-feeding gliders was also sporadic (Goldingay, 1987). There was some evidence that trees were selected for sap feeding according to their ability to produce a consistent sap flow, but no relationship could be found between sap flow and other environmental variables such as soil moisture or rainfall (Goldingay, 1991). In contrast, mountain brushtail possum damage in pine plantations decreased with increasing distance from plantation edges, and was more severe where plantations adjoined the native habitat of possums (Barnett *et al.*, 1977).

Recent 'Plant Vigour' models of herbivory (Coley *et al.*, 1985; Coley, 1988; Price, 1991) suggest that plants growing in resource-rich habitats should be more attractive to herbivores than slower growing plants, due to lower concentrations of secondary defence compounds. Trees growing on higher quality sites also tend to have a higher nutrient status (Lamb, 1977; Bell and Ward, 1984; Judd *et al.*, 1991; Judd *et al.*, 1996) - an obvious benefit to herbivores. Growth rate of eucalypts varies enormously, even in fertilised eucalypt plantations, in response to local variation in soil type, fertility, and water storage capacity (Schönau and Herbert, 1989; Dutkowski, 1995; Hingston and Galbraith, 1998; Hingston *et al.*, 1998; Judd *et al.*, 1996). It was therefore hypothesised that tree growth rate was a significant factor in the distribution of tree damage in plantations of *E. globulus*.

This chapter describes a survey to assess the distribution of tree damage in a number of plantations across the rainfall gradient of the south-west of Western Australia, where plantations of *E. globulus* are being established on a commercial basis. Similar surveys were undertaken by Braithwaite *et al.* (1983), Pauses *et al.* (1995) and Munks *et al.* (1996) to find habitat variables that helped predict density and diversity of arboreal marsupials. Statistical analysis of damage and site data collected in surveys has led to a greater understanding of the causes of damage and provided useful frameworks for predicting damage (Gill, 1992).

The current survey was designed to take account of both regional and local differences in damage. Its aims were (i) to examine patterns of damage to plantations, (ii) to identify variables that best explain the distribution and intensity of damage, and (iii) to develop a conceptual model to predict areas at high risk of damage. This model will serve as a framework on which to base hypotheses for further research. *Barnardius zonarius* is present throughout the region, with varying proportions of the subspecies (*B. z. zonarius* and *B. z. semitorquatus*) and their hybrids (see Section 1.2). It was assumed that both subspecies were capable of damaging *E. globulus*, and no attempt was made to differentiate between them during this survey.

2.2 Methods

2.2.1 Survey design

Twelve plantations of two-year-old *E. globulus* were selected from those established by one company, Bunnings Treefarms (Fig. 2.1). One compartment was surveyed in each of seven plantations, while two compartments were surveyed in each of the other five plantations, giving a total of 17 compartments (Table 2.1). All plantations surveyed were established in 1993 on existing farmland using identical site preparation and planting techniques. Trees were planted 2 m apart in rows 4 m wide, giving a potential stocking of 1250 stems ha^{-1}. Compartments selected varied in size from 7.8 ha to 59.5 ha and, in some compartments, only a portion of the area was surveyed. The plantations represented the range of climatic conditions over which *E. globulus* is grown in Western Australia, from approximately 650 to 1400 mm mean annual rainfall (Fig.2.1), and included a variety of soil and landscape positions. The dominant eucalypt species in the native vegetation varied across the region (see Fig. 1.1).

Damage was assessed at approximately 100 m intervals across compartments in sampling plots consisting of 15 trees (five trees x three rows), giving a sampling intensity of approximately 1.2 %. Care was taken not to over-represent edge plots. In most plantations, tree rows were established along contour lines rather than in straight lines, so that the location of sampling plots varied slightly from a strict 100 m x 100 m grid. Distance between plots was measured by pacing, after calibration with a hip chain, and the position of each plot recorded on aerial photographs and plantation maps. Plot trees were marked with surveyor's tape and numbered for future reference. A total of 465 plots (6975 trees) were assessed for tree damage. Selected plantations were assessed in random order in winter of 1995 (13/6/95 - 14/8/95). Parrot activity causing damage was reportedly at its lowest during winter (Ritson, 1995), and parrots were not expected to cause additional damage during the assessment period (see pp11-12).

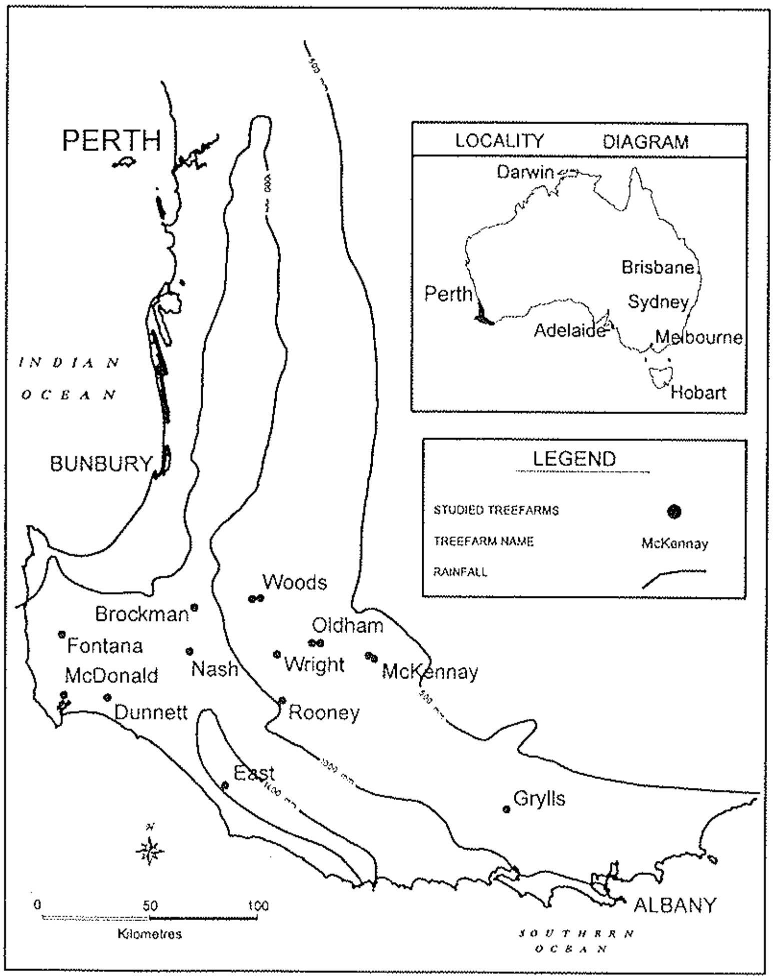


Figure 2.1 Location of the twelve plantations of two-year-old *Eucalyptus globulus* surveyed for parrot damage in the south-west of Western Australia. Five plantations (McKennay, Oldham, Wood, Nash and East) each had two compartments surveyed, while only one compartment was surveyed in the remaining seven plantations.

Table 2.1.
Location (eastings E, northings N) of plantations (and compartment number) surveyed for tree damage. The number of grid plots (n) per compartment is shown.

Shire	Plantation/ Compartment	Number of grid plots	Grid reference
Augusta-Margaret River	McDonald 5	16	E332163 N6208750
Augusta-Margaret River	Fontana 1	22	E330073 N6237116
Nannup	Dunnet 1	33	E351623 N6208916
Nannup	Nash 2	15	E389369 N6229804
Nannup	Nash 3	10	E388959 N6230697
Nannup	Brockman 2	25	E391430 N6250083
Manjimup	East 1	9	E407365 N6165203
Manjimup	East 2	20	E407042 N6167456
Manjimup	Rooney 1	26	E432208 N6207699
Bridgetown-Greenbushes	Wood 1	28	E421236 N6255780
Bridgetown-Greenbushes	Wood 5	18	E421046 N6254774
Bridgetown-Greenbushes	Wright 1	27	E430010 N6229015
Boyup Brook	Oldham 6	48	E445908 N6234509
Boyup Brook	Oldham 18	64	E449422 N6234150
Boyup Brook	McKennay 1	45	E473095 N6227802
Boyup Brook	McKennay 2	42	E474417 N6227805
Plantagenet	Gryll 2	17	E537328 N6157550

2.2.2 Dependent variables to measure parrot damage

1. *Strip Length* - Cumulative length of bark stripped from the central stem of each tree was estimated by eye to the nearest 10 cm after an initial calibration period. Lengths less than 5 cm were not recorded. Stripping was classified and recorded as being either 'old' or 'recent' according to whether or not the wound had callused significantly. Total strip length per plot (TSL) was calculated as the sum of lengths of bark stripped from the central stem of each tree.
The length of bark stripped was assumed to be proportional to the attractiveness of the tree. A short strip length could result from a disturbance to the feeding episode, rather than to the attractiveness of the tree, but this effect was probably minor. TSL provides a measure of the intensity or severity of damage per plot and takes into account different strip lengths per tree. Trees with broken tops as a result of ringbarking by parrots were recorded and the length of stripping on the broken top included wherever possible.

2. *Percent Tree Damage* - The proportion of trees damaged per plot was calculated to give a measure of the incidence of damage (TD).

2.2.3 Independent variables influencing parrot damage

Several independent variables were measured during the survey, while others were derived from interpretation of aerial photography (scale 1:10,000) and climatic data. Some variables were relevant only at the plantation scale (e.g. parrot abundance, rainfall, area of surrounding vegetation) but provided a useful basis to make comparisons of damage across the region. Other variables were measured for each plot, either as continuous or as categorical data, and these allowed more detailed analysis of differences in damage both between and within plantations. Variables can be broadly grouped into those related to growth of plantation trees and those related to parrot habitat and abundance. Some variables might have influenced both trees and parrots.

1. *Diameter of each tree at breast height* (DBH -1.3 m above ground) is commonly used as a surrogate measure of stem volume and biomass of plantation trees (Husch *et al.*, 1993) and was used in this thesis to give an estimate of 'tree growth' for comparative purposes (see also Fig. 3.2). Diameter growth was not significantly affected by parrot damage (see Table 3.1). Tree height was not used because of the large number of trees that had broken tops as a result of tree damage. The growth phase of each tree, either juvenile (all leaves in the juvenile phase) or adult (true adult leaves present) was recorded. Mean plot DBH was used for inter-plot comparisons, while DBH of individual trees was used for some inter-tree comparisons.

2. *Surface soil type* was described for each plot (McDonald *et al.*, 1990), and later classified into one of six soil types, based loosely on the soil groups of Western Australia (Tille, 1996; Schoknecht, 1997) with attributes related to water and nutrient holding capacity:
S - sandy topsoil, usually white or grey, including wet sands, <20 % ironstone gravel, usually found along poorly drained watercourses and on coastal plains
SG - sandy gravel with >20 % ironstone gravel, white (bleached) or grey loose sandy matrix, often coarse, and usually associated with laterite formed over sedimentary materials, on crests and upper to mid-slopes
LG - loamy gravel with >20 % ironstone gravel, yellow or brown loamy matrix, usually associated with laterite and occurring on the lateritic plateau and upper slopes
RBC - red-brown clay, including red-brown gravels and red-brown loams, commonly found in dissected terrain on fresh rock, on side-slopes and footslopes, and often associated with dolerite dykes
HG - heavy gravel with >60 % ironstone gravel, including shallow, stoney and skeletal soils, usually found on laterite outcrops, crests and upper slopes in association with yellow-brown gravels and bleached gravelly sands
SS - salty sands and clays along drainage lines and valley flats, indicated by bare soil, barley grass (*Hordeum geniculatum*), sedges and salt-water couch (*Cynodon dactylon*), usually found in areas with rainfall less than 900 mm, subsoil often waterlogged in winter.

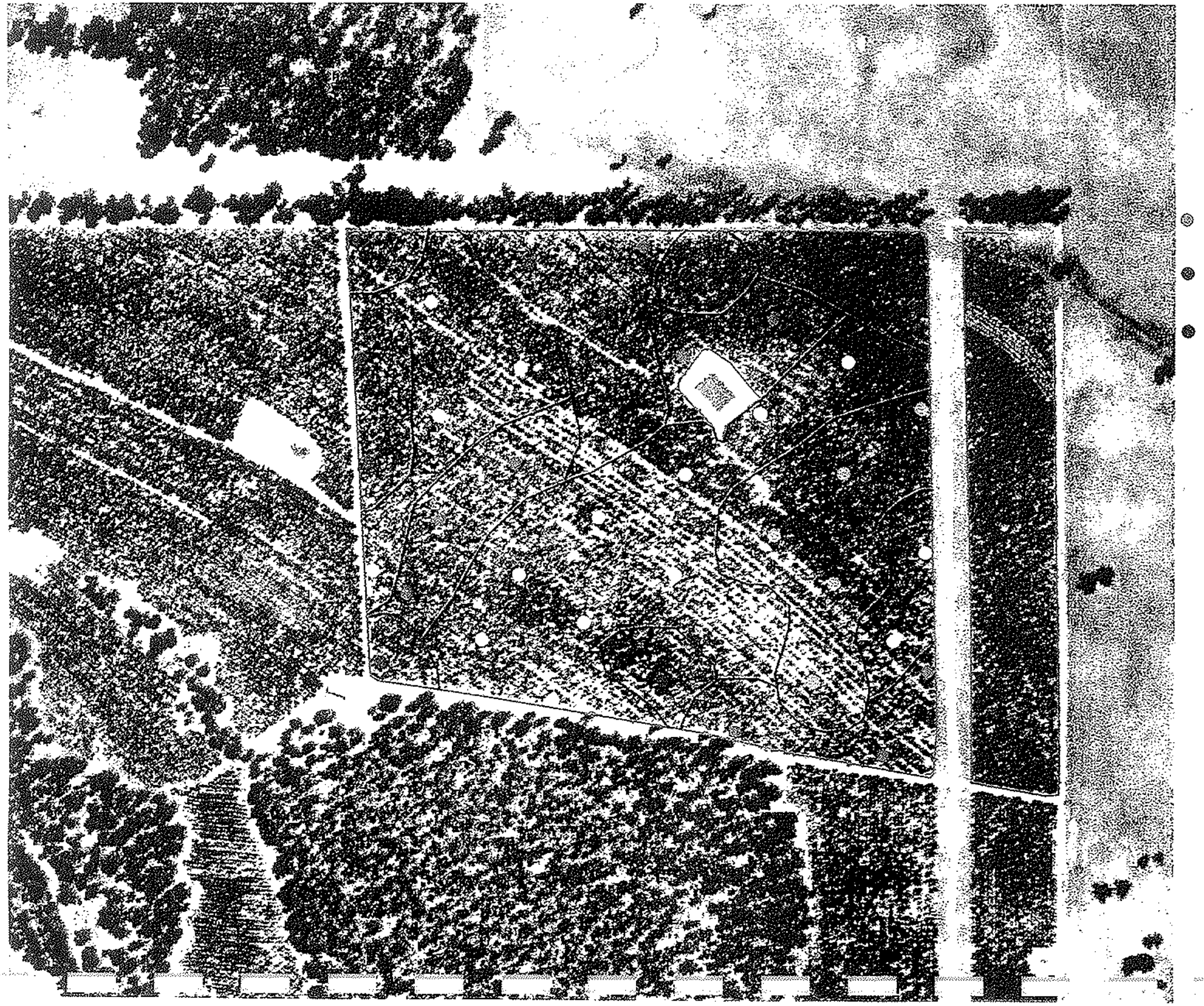
3. *Landscape position* for each plot within a toposequence was determined in the field and confirmed from air photo interpretation, and from 1:50,000 maps with 10 m contours according to McDonald *et al.*'s (1990) categories:

C - hillcrest
U - upper slope
M - mid-slope
L - lower slope
F - flat adjacent to open depression or creekline
D - open depression or vale, usually mid-slope

Aerial photographs (1:10,000) of each compartment were scanned and the data captured and digitised in the Australian Map Grid, Zone 50. Accurate co-ordinates of up to 30 visible points on each aerial photograph were determined in the field using a sub-meter Global Positioning System (GPS). These were then used to correct the aerial photographs to geographic co-ordinates using an imaging module of the Integraph MGE suite (1997). Internal vegetation features were subsequently captured from the corrected image by vectorising boundaries, with areas calculated from digitised line work. Length of edge (internal plus external) and plantation area of each compartment surveyed were determined, and the ratio of length of edge:area (LE:A) calculated. The position of each grid plot was located on these images and colour coded according to tree damage (0, 1 - 25 %, 26 - 50 %, 51 - 75 % and 76 - 100 % TD) (e.g. Fig. 2.2). Colour maps of each compartment were produced showing areas of Nil, Moderate and High tree damage, corresponding to zero, 1 - 50 %, and ≥ 50 % TD, respectively. Boundaries between these damage zones were drawn using a digitiser on the coded aerial photograph, usually mid-way between relevant grid plots, but with some subjective interpretation based on ground knowledge (Fig. 2.2).

MCKENNAY FOREST
CMPT 2 P1993

BUNNINGS
TREEFARMS



Zero Damage
1 - 25% Trees damaged
● 26 - 50% Trees damaged
● 51 - 75% Trees damaged
● 76 - 100% Trees damaged

Figure 2.2 An example of a digitised aerial photograph of the McKennay 2 plantation. Plots of trees surveyed for parrot damage are shown with colour-coded dots representing the five categories of parrot damage. Areas of similar damage were defined by drawing boundaries approximately mid-way between damage zones.

Several additional independent variables were derived from these digitised images as follows:

5. *Distance to the nearest edge* (DE). The distance (m ± 5) from each plot to the nearest edge or boundary of the compartment was estimated from digitised images. Edges around internal remnants of native vegetation or creeklines and large areas of rocky outcrops left unplanted, were included as well as compartment boundaries. Edges around small internal discontinuities or single native trees within compartments were not considered.

6. *Distance to the nearest mature native trees* (DMNT) Digitised images were also used to calculate the minimum flight distance (m ± 5) from habitat trees to plot trees. Mature native trees were those that would be suitable for use as habitat trees by parrots, whether for nesting, feeding, perching or shade. Isolated individual native trees were not considered in this calculation.

7. *Edge type* (ET) was classified according to the presence and size of patches of mature native trees, parts of which were within 100 m of the nearest edge for each grid plot. Categories of edge types were:
1 - open paddock, no mature native trees
2 - scattered mature native trees, discontinuous
3 - linear creekline vegetation of mature native trees <100 m wide, continuous
4 - linear road verge or fenceline vegetation of mature native trees <100 m wide, continuous
5 - patch, small remnant of mature native trees, <4 ha area
6 - bush, remnant of mature native trees, 4 - 10 ha area
7 - forest, state or private forest, >10 ha area
8 - plantation, *E. globulus*
9 - other vegetation, e.g. low shrubs, regrowth, pine plantation

8. *Parrot abundance* (PA) was assessed for each compartment during the survey by marking on plantation maps the presence of parrots in vegetation surrounding the compartment. Parrots were nesting throughout the survey period with much noisy chattering associated with defending nest hollows (see pp 14-15). This activity was used to subjectively assess the abundance of parrots and to identify their preferred nesting trees.

PA1 - low, few parrots seen or heard

PA2 - moderate, some parrots seen or heard, usually in a restricted location

PA3 - high, many parrots seen and heard, with a relatively even distribution around the compartment

9. *Land Use* within a 10 km x 10 km square surrounding the compartment surveyed was classified into state forest, pine plantation, *E. globulus* plantation, farmland or wetland and the area of each class was calculated from 1:50,000 maps (Department of Conservation and Land Management), using a dot grid acetate overlay (4 ha resolution).

10. *Mean annual rainfall (MAR, mm), annual pan evaporation (E, mm) and relative humidity (RH, %)* for each plantation were interpolated from available (Meteorology Bureau) long-term climate data

2.2.4 Analysis of data

Data were analysed using the analysis of variance (ANOVA), linear regression and generalised linear regression modeling techniques (GLM) in the SPSS 7.5 computer program. Differences in parrot damage among plantations on a regional basis were related to mean annual rainfall, parrot abundance, area of compartment, length of edge:area of compartment, and surrounding land use using linear regression. For analyses at this scale, only one value of the independent variable per compartment was determined (n = 17). Differences in damage among classes for categorical data (e.g. edge type, landscape position and soil type) for all plots were identified using Tukey's test. Data from all plots with equivalent parrot abundance were grouped together to examine the effects of independent variables on damage where there was a similar potential for damage.

Linear relationships between damage and DBH, DE and DMNT for all plots, and for individual compartments, were examined individually and in combination by regression analysis, where the significance of the F statistic (*P*) tested for dependence of Y on X (ie slope of line β). The use of both continuous and categorical data (factors) required a mixed modeling approach. General factorial regression analysis in GLM was used whenever categorical data were involved. Backwards elimination of variables was used for model building with SPSS, based on rejection of variables whose partial regression coefficients had Student *t* values less than the critical value ($\alpha = 0.05$) (Zar, 1996). Interactive terms were included where relevant. Data were subsequently modeled by using the S PLUS computer program. After analysis of the distribution of residuals, data for the independent variable, TD, were square root transformed to improve homoscedasticity. No other data transformations were considered necessary. Backwards elimination of variables was again utilised for the development of a general linear model.

2.3 Results

Regression analysis suggested the two measures of damage, total strip length (TSL) and proportion of trees damaged (TD), were highly correlated ($r^2 = 0.80$, $P<0.001$) for all plots ($n = 465$). This relationship is shown in Fig. 2.3 using mean compartment values. Since TD was more easily measured, and more likely to be adopted by plantation managers, it was used as the dependent variable to develop models of tree damage.

More than 33 % of all trees surveyed had some tree damage with an overall mean strip length per tree of 10.6 cm. Strip lengths greater than 100 cm per tree were not uncommon in severely damaged plots.

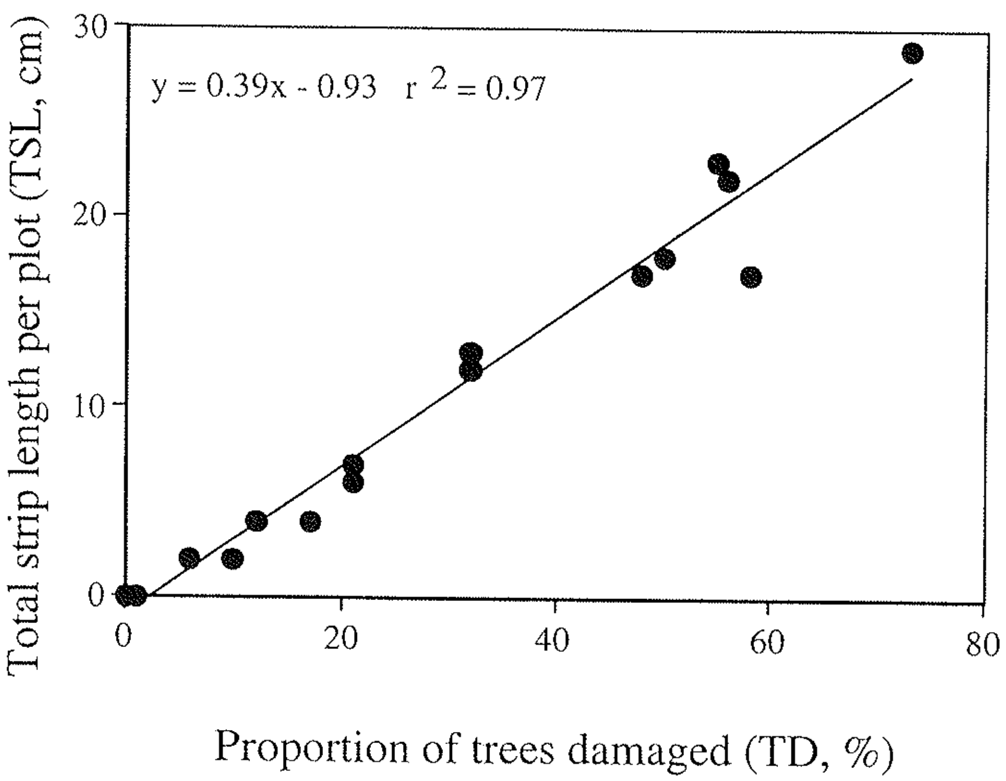


Figure 2.3 Linear regression between mean compartment values (n = 17) of total strip length per plot and the proportion of trees damaged per plot.

Mean compartment TD varied from zero to 73 %. Four compartments had no undamaged plots (Fontana, Wood 5, McKennay 1, Gryll), and five compartments had plots with TD ranging from zero to 100 %. In many plots, every tree had been ringbarked and had broken tops as a result of parrot damage. Replacement leaders (side branches assuming apical dominance after loss of central leader) were also damaged. In the largest compartment surveyed (Oldham 18), 28 % of all trees surveyed (n = 960) had broken tops, while 44 % of trees had strip lengths greater than 10 cm. Old and recent damage was often recorded on the same tree, indicating repeated attacks.

Trees with adult foliage were damaged more frequently than trees still in the juvenile phase. Of all trees surveyed at Oldham 18 (n = 980), 60 % were in the adult growth phase and 68 % of those were damaged, whereas only 36 % of trees in the juvenile phase were damaged. Using single factor ANOVA, there was a highly significant difference between mean strip length (F = 59.5, $P<0.001$) of adult (29.3 cm) and juvenile (14.1 cm) trees, and between DBH (F = 321.2, $P<0.001$) of adult (5.9 cm) and juvenile (4.1 cm) trees in this compartment. Thus trees with adult leaves present were larger and more damaged than trees with all juvenile foliage.

2.3.1 Regional variation in damage

Parrot abundance, Land use and Rainfall Mean compartment tree damage was strongly related to parrot abundance ($r^2 = 0.78$, $P<0.001$, n = 17, see Table 2.2). Mean damage for compartments with PA1 was 4.2 %, compared with 50.5 % for compartments with PA3. PA decreased with increasing mean annual rainfall (MAR) ($r^2 = 0.441$, $P<0.01$), and increased with the proportion of pasture in the 100 km^2 surrounding the plantation ($r^2 = 0.289$, $P<0.05$). Consequently, TD was also negatively related to MAR ($r^2 = 0.264$, $P<0.05$) and positively related to the proportion of farmland surrounding the plantation ($r^2 = 0.303$, $P<0.05$). Thus TD was greatest in drier cereal growing region to the east of the main jarrah (*E. marginata*) forest (e.g. Oldham, McKennay, Gryll), where the majority of land (up to 80 %) was used for farming and where smooth-barked wandoo (*E. wandoo*) woodlands are common (see Fig. 1.1).

Table 2.2
Mean plot tree damage (TD), parrot abundance (PA), mean plot diameter at breast height (DBH), mean annual rainfall (MAR), surrounding land use (proportion of farmland), area of compartment, and the ratio of length of edge to area of compartment (LE:A) for seventeen plantation compartments surveyed in south-west Western Australia.

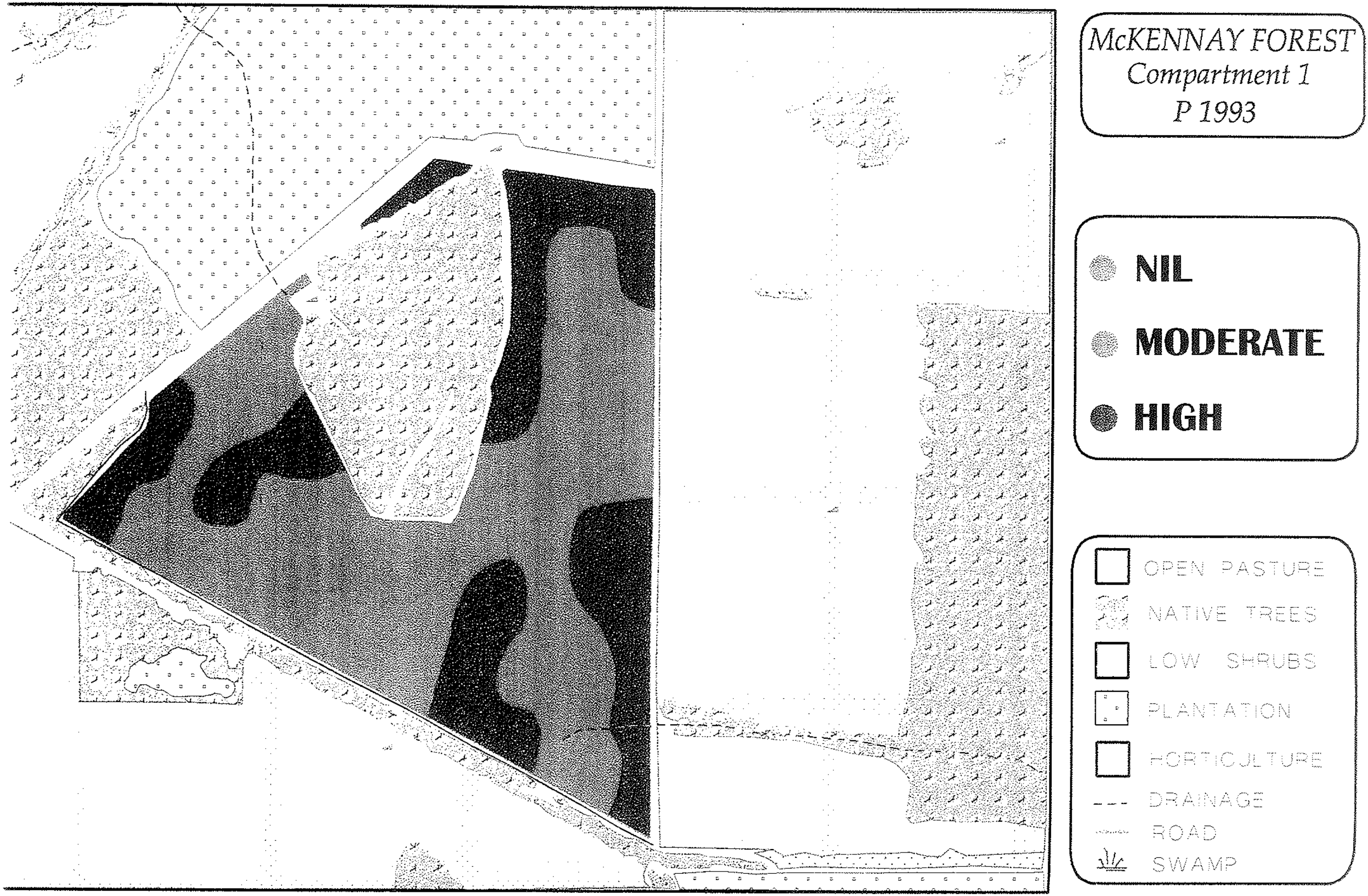
Plantation / compartment	TD (%)	PA	MAR (mm)	Land-use (% farmland)	Area (ha)	LE:A (m ha^{-1})	DBH (cm)
McKennay 2	32	3	649	81	28	74	4.8
McKennay 1	48	3	649	81	45.3	97	5.4
Oldham 6	32	3	685	63	30.7	77	4.9
Oldham 18	55	3	685	63	59.5	88	5.1
Gryll 2	56	3	796	97	22	94	8.5
Wright 1	50	3	876	42	22.8	107	6.0
Wood 1	21	2	903	73	24	127	6.4
Wood 5	58	3	903	73	19.4	166	6.3
Rooney 1	6	1	1005	37	36.2	106	6.9
Brockman 2	12	2	1082	33	27.3	130	7.3
Dunnet 1	21	2	1096	80	20.8	103	7.2
McDonalds 5	0	1	1097	70	35.5	81	7.6
Nash 3	10	1	1121	45	12.1	117	7.9
Nash 2	12	2	1121	45	7.8	187	7.2
Fontana 1	73	3	1165	72	13.8	119	6.9
East 1	1	1	1432	30	29	80	7.4
East 2	17	2	1432	30	38.6	85	7.0

Despite the overall negative relationship between rainfall and tree damage, considerable damage was found at Fontana plantation near Margaret River, on the western edge of the jarrah forest where mean annual rainfall was 1165 mm. This site suffered the most damage of all plantations - some 73 % of surveyed trees were damaged with a mean total strip length of 29 cm per plot. Parrots were abundant in this area and were possibly attracted by the establishment of many new vineyards and other horticultural crops. Within the jarrah and karri (*E. diversicolor*) forest regions (e.g. Rooney, Nash, Brockman), where the proportion of farmland was low (38 %), and along the south coast region (e.g. McDonald, Dunnet, East), tree damage was generally low.

No correlation between DBH and TD was apparent across mean annual rainfall (MAR) regions (Table 2.2). This was most likely a result of the negative relationship between PA and MAR ($r^2 = 0.441$) counteracting the positive relationship between MAR and DBH ($r^2 = 0.436$, $P<0.01$). Thus, while diameter at breast height increased with increasing mean annual rainfall, parrot abundance and tree damage decreased. Tree damage was unrelated to area of compartment, ratio of length of edge to area of compartment (Table 2.2), relative humidity, or land use (other than farmland).

2.3.2 Variation in damage between compartments within plantations

Length of Edge:Area In four of the five plantations in which two compartments were surveyed, damage differed significantly between the compartments (Table 2.3), even where parrot abundance was equivalent (i.e. McKennay and Oldham). In four compartments showing significant variation (McKennay, Oldham, Wood, East), greater damage corresponded with a greater edge to area ratio (Table 2.2), but this was not always due to smaller compartment size. For example, in McKennay 1, a large internal remnant of mature native trees added 1520 m of edge to the compartment, increasing the length of edge by more than 50 % (Fig. 2.4). Similarly, at Wood 5, the internal remnant increased LE by 24 %.



McKENNAY FOREST
Compartment 1
P 1993

BUNNINGS
TREEFARMS

FS 9735-5B

PARROT DAMAGE ASSESSMENT

Figure 2.4 Parrot damage in the McKennay 1 plantation showing the effect of a large internal remnant of native vegetation on the level of damage. Parrots were very active in this remnant and caused severe damage to adjacent plantation trees.

Table 2.3
Summary of one-way ANOVA of tree damage (TD) between two compartments in each of five plantations.

Plantation	F	*P* value
McKennay	9.313	0.003
Oldham	17.058	<0.001
Wood	23.824	<0.001
East	7.901	0.009
Nash	0.036	ns

At Wood 5 and East 2, increased tree damage was also associated with greater parrot abundance. Although approximately half of the compartment edge of Wood 1 was adjacent to native forest, parrots were only active in one area, whereas in Wood 5, almost all edges were adjacent to mature native trees in which parrots were active. At East 1, the only remnant of native vegetation adjacent to the compartment was young regrowth karri.

2.3.3 Variation in damage among all plots

Across all plots surveyed (n = 465), TD varied from zero to 100 %. Maximum TSL was 1190 cm for one plot at Oldham 18 (i.e. mean strip length of 79 cm per tree).

Edge type The type of edge adjacent to compartments had a small but significant effect ($r^2 = 0.123$, $P<0.001$) on the amount of damage to plantation trees (Table 2.4a). Damage was greatest where edges were linear remnants of mature native trees, either in road verges or in creekline vegetation, and where scattered mature native trees were left in otherwise cleared paddocks. Damage was least where plantation trees were adjacent to other plantation compartments, to large forest blocks, or to immature remnant vegetation (i.e. 'other'). When edge type and landscape position were combined in a GLM, they explained 54 % of the variation in parrot abundance. When mean annual rainfall was included in this model, 70 % of the variation was explained. Edge type can be used to qualify estimates of parrot abundance on a local scale.

When plots with high parrot abundance were considered together, ranking of edge type changed. Plots adjacent to 'scattered trees' had the greatest damage (80 %), while those adjacent to another plantation compartment ranked fourth (48 %). Inadequate replication for some edge types in separate parrot abundance categories reduced the value of these comparisons. For example, there were no cases of 'linear road verge', 'linear creekline' or 'scattered tree' categories for compartments where parrot abundance was low, and no 'other' edges where parrot abundance was high.

Landscape position Topographic position of plantation trees had some influence on the level of tree damage ($r^2 = 0.098$, $P<0.001$). Trees growing in open depressions and on hillcrests were damaged more than those growing on flats (Table 2.4b). Compartments in which trees growing in higher parts of the landscape were more severely damaged included Oldham 6, Oldham 18, Brockman 2, Gryll 2, Rooney 1 and Wood 1. There was a highly significant but weak effect of landscape position on DBH ($r^2 = 0.056$, $P<0.001$). Growth rate of trees is generally greater on crests and upper slopes in these landscapes (Darling Plateau) where lateritic soil profiles are deep. However, at the Fontana plantation, the reverse pattern was observed. Tree growth was markedly reduced at hillcrest locations where hard lateritic caprock lay close to the soil surface. Here, damage to trees was minimal while on the lower slopes, adjacent to a fresh water creekline, trees were large and were severely damaged by parrots.

Table 2.4

Effect of a) edge type, b) landscape position, and c) soil surface type on mean tree damage (TD) and parrot abundance (PA) for all plots (n = 465). Edges were classed according to the presence and size of patches of mature native trees within 100 m of the nearest edge for each plot. See methods for definition of each edge type. Means followed by the same letter are not significantly different ($P<0.05$, Tukey's test). nd = not determined.

Parameter	TD	PA	n
a) Edge type			
Linear road verge	52.4 a	2.80	100
Linear creekline	45.3 ab	3.00	48
Scattered trees	37.5 abc	1.89	19
Patch	30.2 bc	2.15	40
Bush	29.7 bc	2.54	69
Open	27.8 bc	2.50	24
Plantation	24.5 c	2.34	112
Forest	23.8 c	1.81	37
Other	13.8 c	1.50	16
b) Landscape position			
Open depression	53.1 a	2.91	23
Hillcrest	48.5 a	2.64	53
Lower slope	39.8 ab	2.86	58
Upper slope	36.9 ab	2.50	70
Mid-slope	33.7 b	2.57	150
Flat	19.4 c	1.79	111
c) Soil surface type			
Red-brown clay	44.3 a	nd	66
Sandy gravel	37.7 a	nd	44
Loamy gravel	37.6 a	nd	214
Salty sand	28.0 ab	nd	9
Heavy gravel	27.7 ab	nd	12
Sand	22.3 b	nd	120

Landscape position was, however, confounded with parrot abundance (Table 2.4b) ($r^2 = 0.233$, $P<0.001$). Most plots from south coast plantations, with low parrot abundance, were categorised as flats (i.e. coastal plains). When plots with high parrot abundance (PA3) were considered together (n = 287), trees growing on crests and open depressions were still damaged slightly more than other plots ($r^2 = 0.044$. $P<0.01$). For plots with high parrot abundance, DBH was also related to landscape position ($r^2 = 0.22$, $P<0.001$), being greater in upland positions and least on lower slopes and flats (Table 2.5)

Surface soil type There was little effect of surface soil type on tree damage ($r^2 = 0.050$, $P<0.001$). Trees growing on red-brown clay, sandy gravel and loamy gravel were more severely damaged than those growing on sand (Table 2.4c). Soils at most south coast plots (McDonald, East, Dunnet) were classed as sand and the effect of soil type was again confounded with parrot abundance. If plots with high parrot abundance (PA3) were considered separately, TD was significantly greater for trees growing on red-brown clay (62 % damaged). Surface soil type also had a significant effect on DBH ($r^2 = 0.165$, $P<0.001$) for trees in this PA3 category - DBH was greatest on red-brown clays (6.2 cm) and loamy gravels (5.9 cm), and least on salty sands (3.3 cm).

Clearly, parrot abundance, mean annual rainfall, edge type, landscape position and soil type frequently interact in their effects on parrot damage. Tree growth rate (DBH) could be predicted using a mixed model approach (GLM) including mean annual rainfall, landscape position and soil type ($F = 13.49$, $r^2 = 0.412$, $P<0.001$), and parrot abundance could be predicted by mean annual rainfall, landscape position and edge type ($F = 24.65$, $r^2 = 0.696$, $P<0.001$).

The extent to which factors related to parrot damage reflect differences in parrot abundance, or differences in tree growth rate *per se*, was not readily apparent. A direct relationship between tree growth (as measured by DBH) and TD was most clearly demonstrated when differences in damage within compartments were examined. At this scale interacting factors, such as mean annual rainfall and parrot abundance, were relatively constant.

Table 2.5
Effect of landscape position on the proportion of trees damaged (TD) and mean DBH for plots where parrot abundance was high (n = 287). Means followed by the same letter are not significantly different (*P*<0.05, Tukey's test).

Landscape position	TD %	DBH cm	n
Hillcrest	59.9 a	6.3	36
Open depression	57.6 ab	5.8	21
Upper slope	55.4 ab	6.1	42
Mid-slope	45.0 ab	5.9	105
Lower slope	41.6 b	4.9	52
Flat	36.7 b	4.0	31

2.3.4 Variation in damage within compartments

The distribution of tree damage within some compartments was positively related to DBH (Table 2.6), while in other compartments, distance to edge (DE) and distance to nearest mature native trees (DMNT) were more important variables (Table 2.7). Only some variables were related to damage in each compartment. Edge type was also important in some compartments.

For those compartments where tree damage was not significantly related to DBH, either:

a) the range of DBH was small, so that a linear relationship was not statistically apparent (e.g. East 1, Nash 2 and 3, Brockman, Gryll),

b) the compartment size was large in relation to the parrot population, or parrot activity was very localized, so that not all trees had an even probability of being damaged (e.g. East 1, Rooney, Brockman, Oldham 6), or

c) the compartment size was small in an area of high parrot abundance, and little selectivity was evident (e.g. Wright).

Diameter at breast height (DBH) Damage was strongly ($P<0.001$) related to mean plot DBH within three compartments (Dunnet, Oldham 18, and Fontana) regardless of mean annual rainfall region (Table 2.6). The relationship was most obvious in these compartments because of the high degree of variability in DBH.

Table 2.6

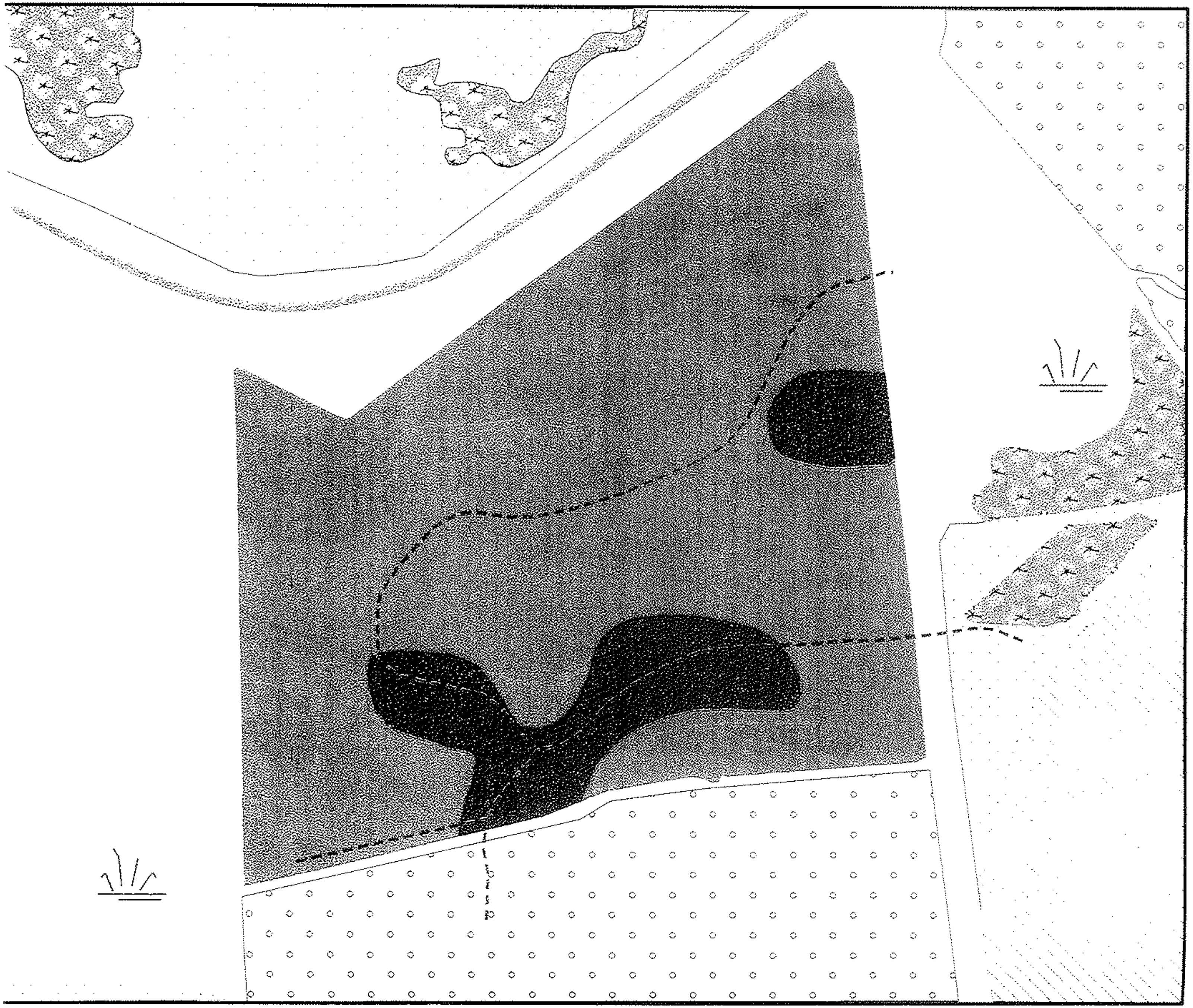
Tree damage (TD) and diameter (DBH, mean and range) for surveyed compartments. The number of grid point plots (n) in each compartment is indicated. The coefficient of determination (r^2) and significance (*P*) of a linear regression between TD and DBH are indicated (**P*<0.05, ***P*< 0.01, ****P*<0.001, ns not significant).

Plantation/ Compartment	n	TD (%)	DBH (cm)		r^2	*P*
			Mean	Range		
McDonalds 5	16	0	7.6	5.7 - 10.2	-	-
East 1	9	1	7.4	5.0 - 9.1	0.010	ns
Rooney 1	25	6	6.9	3.9 - 8.7	0.040	ns
Nash 3	10	10	7.9	6.9 - 9.3	0.123	ns
Nash 2	15	12	7.2	5.6 - 9.2	0.004	ns
Brockman 2	25	12	7.3	5.0 - 9.8	0.112	ns
East 2	20	17	7.0	3.3 - 10.8	0.290	*
Dunnet 1	33	21	7.2	2.2 - 10.2	0.626	***
Wood 1	28	21	6.4	4.1 - 8.8	0.016	ns
McKennay 2	42	32	4.8	2.8 - 6.8	0.171	**
Oldham 6	48	32	4.9	3.3 - 6.9	0.021	ns
McKennay 1	45	48	5.4	3.4 - 7.4	0.080	ns
Wright 1	27	50	6.0	3.0 - 7.8	0.040	ns
Oldham 18	64	55	5.1	2.2 - 7.4	0.480	***
Gryll 2	18	56	8.5	6.6 - 9.8	0.006	ns
Wood 5	18	58	6.3	5.4 - 7.8	0.251	*
Fontana 1	22	73	6.9	4.1 - 10.1	0.511	***

For example, at the Dunnet plantation (1096 mm MAR), mean plot DBH varied from 2.2 - 10.2 cm due to variation in nutrient availability (N, Cu, Zn and Mn) and seasonal waterlogging associated with the coastal sand dune system. Damage at this plantation was clearly related to DBH ($r^2 = 0.626$, Table 2.6) being most severe in the large trees along drainage lines (80 % TD), whilst one third of the compartment incurred no damage at all (Fig. 2.5). There was no effect of edge type, soil type or landscape position on damage for this compartment, being on flat sandplain country with few mature native trees nearby (mean DMNT = 289 m). Thus the surrounding habitat had little effect on parrot access to plantation trees, and all trees had a similar chance of being damaged.

A further example of the strong relationship between damage and DBH was found at Oldham 18 ($r^2 = 0.480$, Table 2.6). Here, linear corridors of mature native trees surrounded the compartment, conferring a similar potential for damage from any edge (Fig. 2.6). Parrots were active in all edges even though native eucalypt species varied slightly from creekline (*E. rudis*) to road verge (*E. marginata* and *E. wandoo*). Mean plot DBH varied from 2.2 - 7.4 cm, and was limited by mean annual rainfall (685 mm). Analysis of data from this compartment showed that DBH was related to soil type and landscape position ($r^2 = 0.459$ and 0.511, respectively), being greatest on red clay loams and on upper slopes and crests, where soils tend to be deeper. Growth was poorest on saline flats adjacent to creeklines, and where soils were hard and stony and, most likely, quite shallow.

At the Fontana plantation, mean DBH ranged from 4.1 - 10.1 cm and was significantly related to tree damage ($r^2 = 0.511$, Table 2.6). In contrast to Oldham 18, damage was most severe in this compartment in trees adjacent to the fresh water creek where growth was unaffected by salinity. Damage was also related to DBH at McKennay 2, Wood 5 and East 2.



DUNNET FOREST
Compartment 1
P 1993

● NIL
● MODERATE
● HIGH

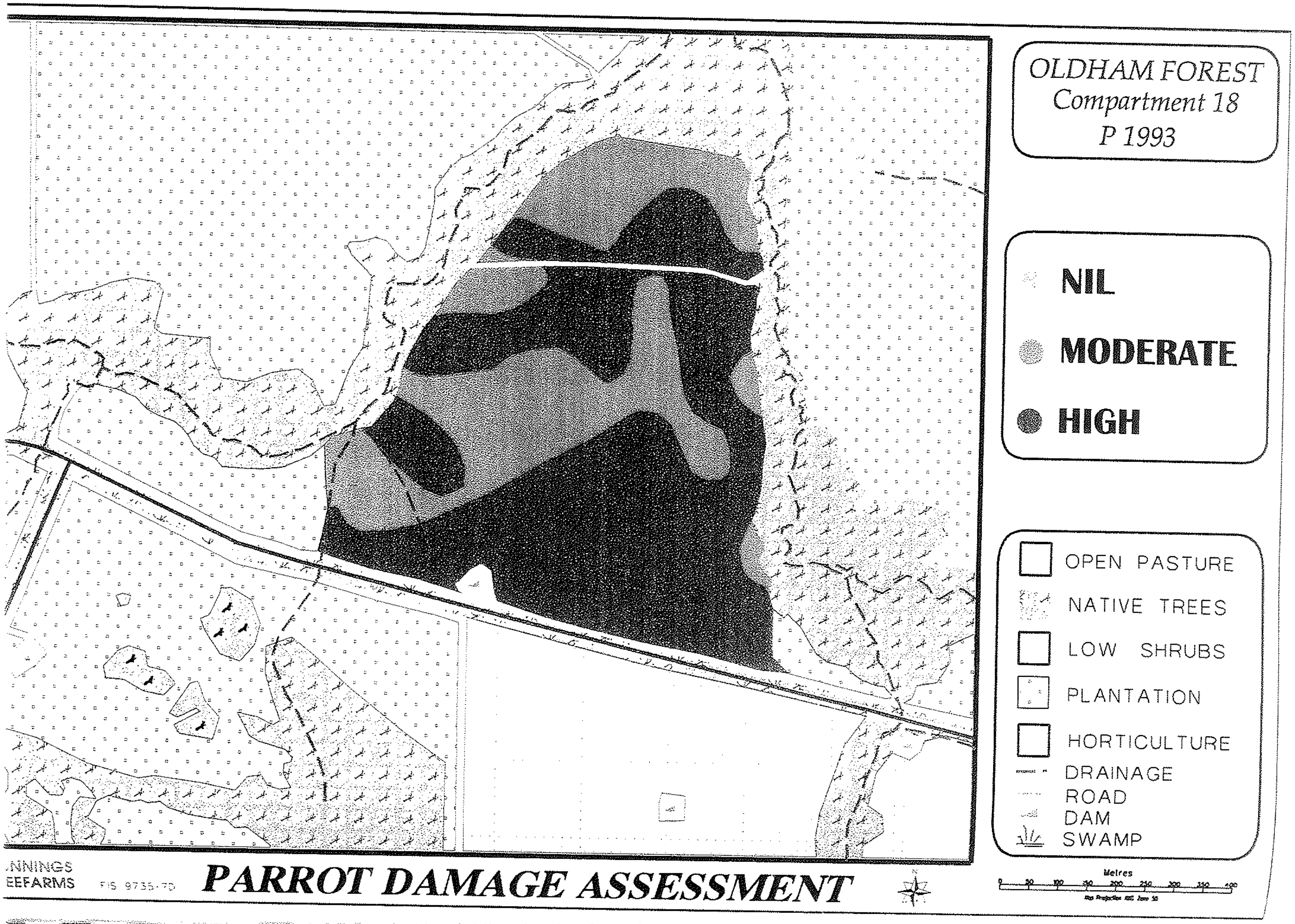
☐ OPEN PASTURE
▨ NATIVE TREES
☐ LOW SHRUBS
▨ PLANTATION
☐ HORTICULTURE
--- DRAINAGE
ROAD
SWAMP

Metres
0 50 100 150 200 250 300
Map Projection AMG Zone 50

BUNNINGS
TREEFARMS
FIS 9736-2B

PARROT DAMAGE ASSESSMENT

Figure 2.5 Parrot damage at the Dunnet plantation showing greatest damage to trees along peaty drainage lines. There was no significant edge effect at this plantation.



PARROT DAMAGE ASSESSMENT

Figure 2.6 Parrot damage at the Oldham 18 plantation, showing high levels of damage throughout the plantation. In this compartment, damage was strongly related to tree growth. There was no edge effect even though parrots were present in all edges.

When all individual trees in the 64 plots measured at Oldham 18 ($n = 980$) were allocated to diameter classes (nine classes i.e. 0 - 2.0, 2.1 - 3.0, 3.1 - 4.0 ... 9.1 - 10.0), to remove the effect of plot location, the proportion of trees damaged in each class increased significantly, and curvilinearly, with DBH ($r^2 = 0.98$, $P<0.001$) (Fig. 2.7).

A similar analysis for Oldham 6 found that DBH explained 93 % of the variation in tree damage, even though the relationship based on mean plot data was not significant. The use of plot means therefore masked some of the variability in the data and resulted in a loss of sensitivity in the relationship between DBH and tree damage. Thus, even within plots, parrots were selectively damaging the larger trees.

Where the relationship between tree damage and DBH was not apparent, it may have been overshadowed by the effects of distance to edge, distance to mature native trees or edge type.

Distance to edge (DE) The effect of DE on damage was clearly illustrated at the Gryll plantation, north of Denmark (Fig. 2.8). In this environment, tree growth was rapid and even throughout the compartment (mean plot DBH ranged from 6.6 - 9.8 cm). All trees surveyed in this compartment were apparently attractive to parrots, as there were no undamaged plots. Damage was most severe in plots adjacent to an edge with mature native trees, and decreased with increasing distance from any edge ($r^2 = 0.642$) (Fig. 2.9).

Distance to nearest edge was also significantly related to tree damage at Oldham 6 (Fig. 2.10), and at McKennay 1 and 2, and to a lesser extent at Dunnet 1 (Table 2.7).

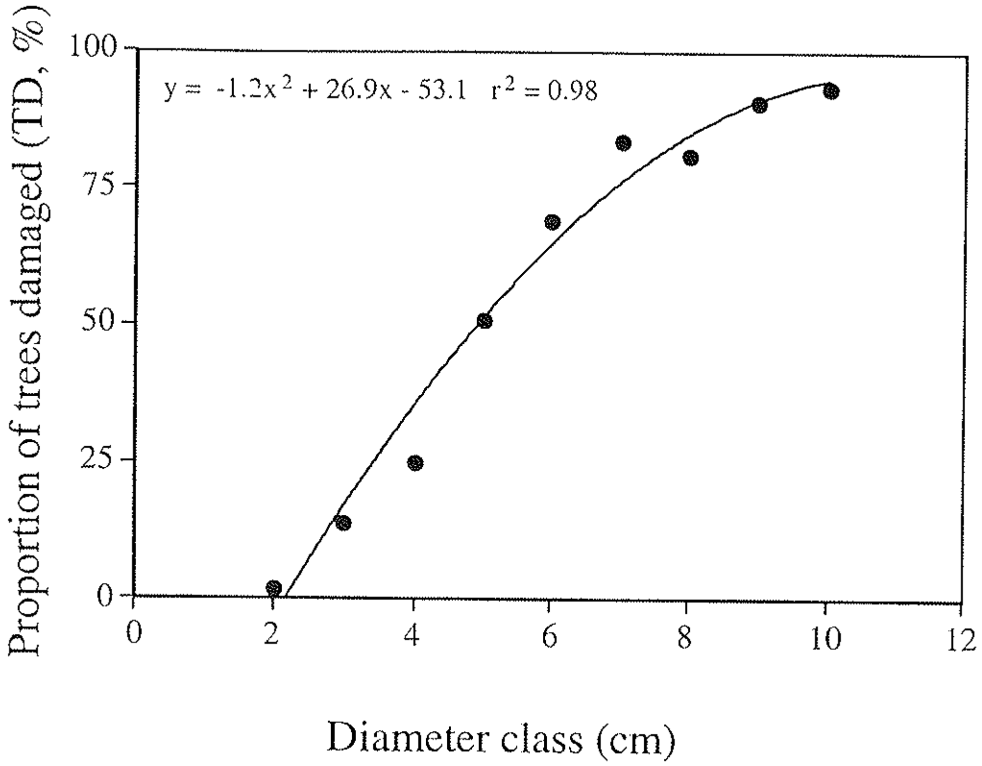
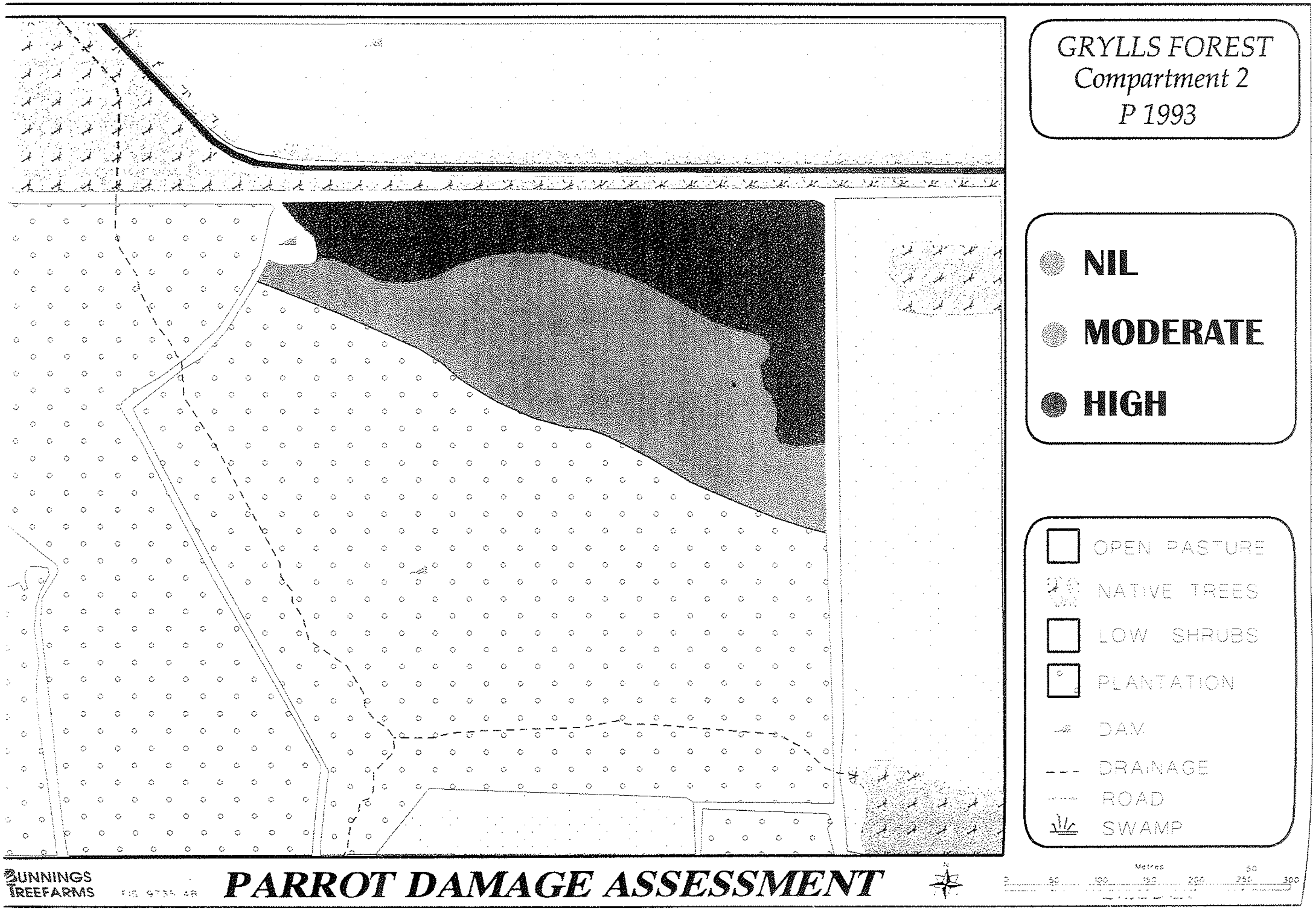


Figure 2.7 The relationship between diameter at breast height (DBH) and the mean proportion of trees damaged in each diameter class (1 cm increments) at the Oldham 18 compartment.



GRYLLS FOREST
Compartment 2
P 1993

PARROT DAMAGE ASSESSMENT

Figure 2.8 Parrot damage at the Gryll plantation showing a strong effect of distance to edge. All trees in this compartment were very well grown and attractive to parrots.

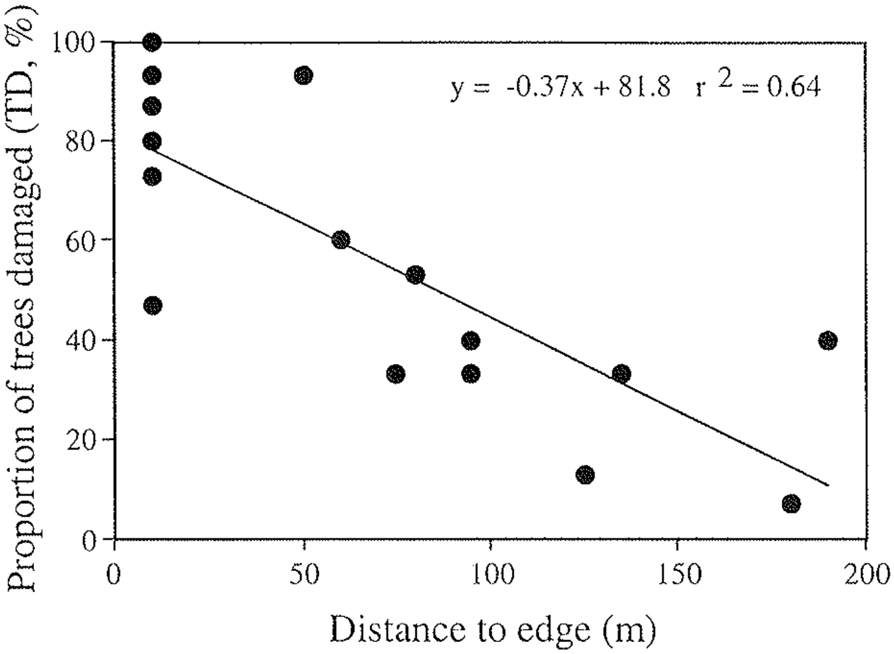


Figure 2.9. Relationship between tree damage and distance of each plot to its nearest edge for the Gryll plantation (n = 17).

Table 2.7
Mean distance to edge (DE) and distance to nearest mature native trees (DMNT) of plots in compartments surveyed. The significance of linear regressions between each variable and the proportion of trees damaged (TD) for plots in each compartment, is shown (**P*<0.05, ***P*<0.01, ****P*<0.001, ns not significant).

Plantation/ Compartment	TD %	DE (m)	*P*	DMNT (m)	*P*
McDonald 5	0	56	ns	611	ns
East 1	1	78	ns	180	ns
Rooney 1	6	40	ns	67	ns
Nash 3	10	36	ns	75	ns
Nash 2	12	25	ns	52	ns
Brockman 2	12	28	ns	143	*
East 2	17	29	ns	59	**
Dunnet 1	21	50	*	289	ns
Wood 1	21	35	ns	91	**
McKennay 2	32	55	**	130	**
Oldham 6	32	65	**	190	***
McKennay 1	48	51	**	98	ns
Wright	50	37	ns	97	ns
Oldham 18	55	71	ns	100	ns
Gryll 2	56	68	***	113	***
Wood 5	58	26	ns	36	ns
Fontana 1	73	34	ns	139	ns

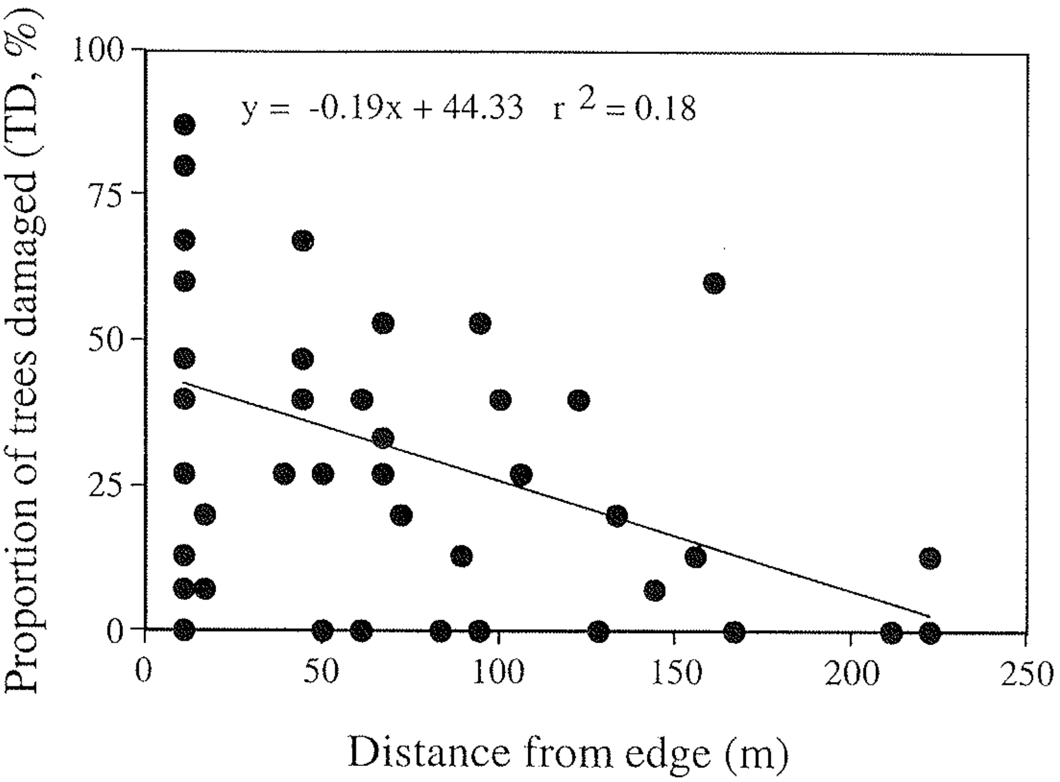


Figure 2.10. The relationship between the distance of each plot to its nearest edge and tree damage for the Oldham 6 plantation (n = 48).

When 'edge' trees within a plantation were defined as those within 50 m of any edge, they were significantly (*P*<0.05) more damaged than 'non-edge' trees in 10 compartments, with no corresponding difference in DBH (Table 2.8). In fact, in no compartments were 'edge' trees significantly larger than 'non-edge' trees. At Wood 1, 'edge' trees had a smaller mean DBH (5.9 cm) than 'non-edge' trees (7.2 cm) due to early establishment problems (insect damage) and suppression of growth by surrounding native forest, yet 'edge' trees were still slightly more damaged. Five other compartments also had smaller 'edge' trees, but the differences in mean DBH between 'edge' and 'non-edge' trees were less significant. Thus distance to edge had a significant effect on tree damage that was not directly attributable to differences in the rate of tree growth.

Small and narrow compartments, and those with a high LE:A (length of edge to area ratio) (Table 2.2), were less likely to show a relationship between DE and damage, due to the low range of DE values. For example, for surveyed plots in Nash 2 and 3, Wood 1 and 5, and Fontana, the mean DE was less than 50 m, but all compartments for which there was a significant relationship between DE and damage had mean DE equal to, or greater, than 50 m (Table 2.8).

An exception to this trend was Oldham 18, which was a large compartment with mean DE of 77 m and low LE:A. In this compartment, parrots were very abundant and trees more than 250 m in from an edge were damaged. The distance that parrots had to fly in order to reach suitable stems probably increased with high parrot abundance. This is based on the assumption that most of the edge trees had already been damaged and there were few undamaged stems left to feed on until replacement lead shoots had time to grow. Where parrot abundance was low (McDonald, East 1, Rooney and Nash 3), damage was recorded only within 60 m of an edge, but in these plantations the number of damaged plots was insufficient to show a relationship with DE.

Table 2.8
Comparison of damage to 'edge' trees (<50 m from edge) and 'non-edge' trees (>50 m from edge) shown by the proportion of trees damaged per plot (TD) and by the total strip length per plot (TSL). Mean diameter at breast height (DBH) for 'edge' and 'non-edge' plots is also shown (n = number of plots)[a]. Significant differences between mean 'edge' and 'non-edge' trees for each compartment are shown (**P*<0.05, ***P*<0.01, ****P*<0.001, ns, not significant).

Plantation		n	TD	*P*	TSL	*P*	DBH	*P*
Gryll 2	edge	7	79.0	***	470	**	8.11	ns
	non-edge	10	40.5		230		8.77	
McKennay 1	edge	23	62.0	***	350	***	5.15	*
	non-edge	22	34.2		163		5.71	
McKennay4	edge	19	47.0	***	298	***	4.77	ns
	non-edge	23	19.2		70		4.78	
Oldham 6	edge	22	41.9	**	254	ns	4.73	*
	non-edge	26	23.8		153		5.10	
East 1&2[b]	edge	16	18.0	**	54	*	7.28	ns
	non-edge	13	5.2		18		6.92	
Nash 2&3[b]	edge	14	18.1	**	77	**	7.19	ns
	non-edge	11	1.8		4		7.78	
Wood 1	edge	16	28.3	*	131	ns	5.86	***
	non-edge	12	11.1		60		7.22	
Brockman 2	edge	14	19.6	*	92	*	7.61	ns
	non-edge	11	1.8		4		6.85	
Rooney 1	edge	12	10.0	*	42	ns	6.50	*
	non-edge	14	1.9		9		7.26	
Wright 1	edge	15	57.3	ns	327	*	5.61	*
	non-edge	12	41.6		202		6.40	
Fontana 1	edge	13	77.9	ns	472	ns	7.17	ns
	non-edge	9	66.7		378		6.51	
Oldham 18	edge	26	54.6	ns	352	ns	4.33	*
	non-edge	38	54.7		345		5.70	
Wood 5	edge	12	59.4	ns	264	ns	6.28	ns
	non-edge	6	55.7		258		6.32	
Dunnet 1	edge	13	22.6	ns	88	ns	7.54	ns
	non-edge	20	20.6		95		7.00	

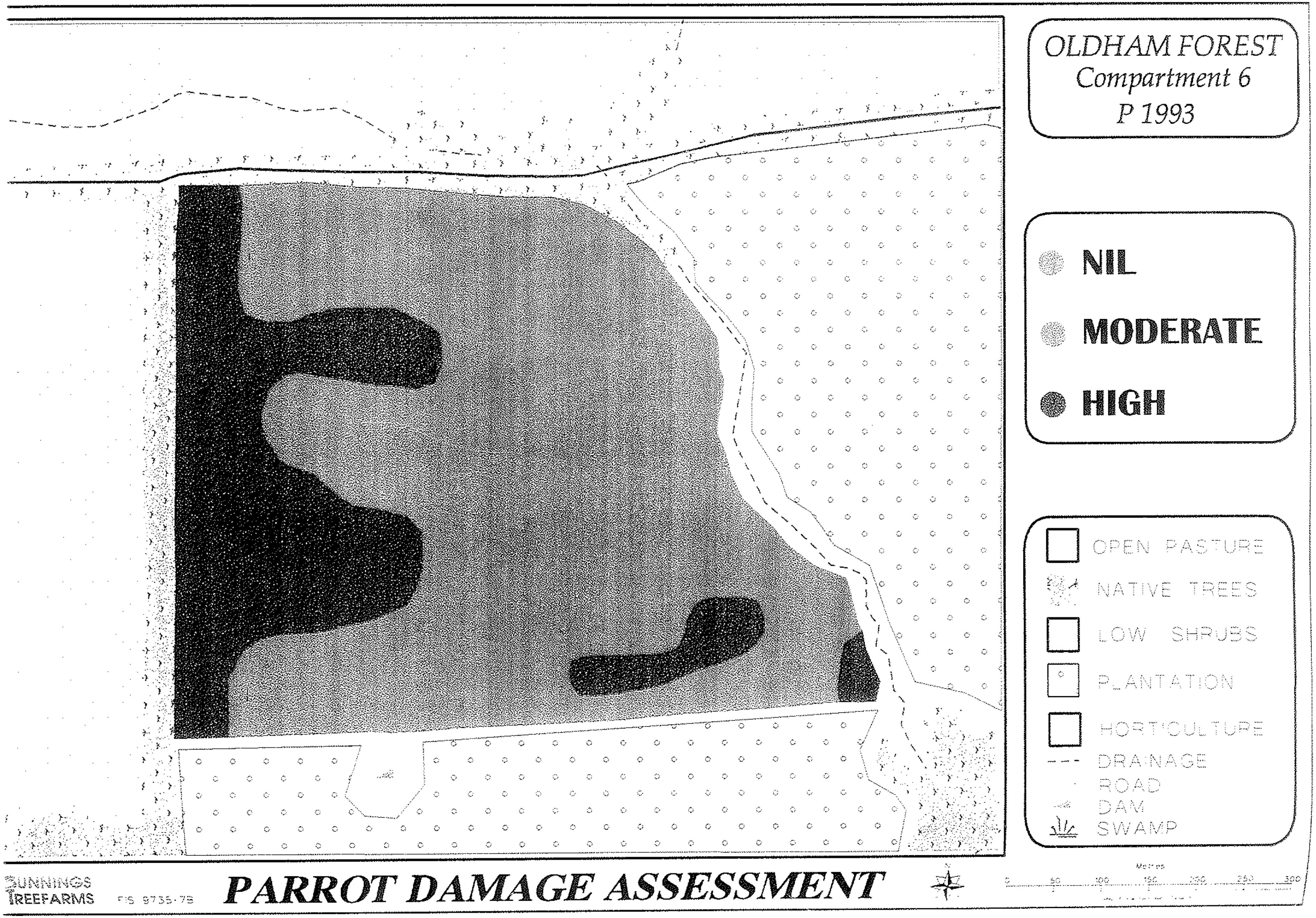
[a] McDonald plantation is not shown in this table as no damage was found.
[b] The two compartments were combined for this analysis.

Distance to nearest mature native trees (DMNT) Damage decreased with increasing DMNT in six compartments surveyed (Table 2.7). Within some compartments, DMNT was a more powerful variable in explaining tree damage than DE. For example, although Oldham 6 had a lower mean DE than Oldham 18 (65 m *c.f.* 71 m), its mean DMNT was almost double that at Oldham 18 (190 m *c.f.* 100 m), corresponding with less overall damage in Oldham 6. Oldham 18 was surrounded by native vegetation whereas at Oldham 6, native vegetation adjoined only two sides (north and west) of the compartment (Fig. 2.11). Damage to trees in the south-east corner of this compartment was the result of parrots entering the plantation from the large remnant of wandoo nearby. Similar comparisons between compartments at both Wood and McKennay show that damage was least in the compartment with the greater mean DMNT.

Mean DMNT for all plots in all plantations was 145 m compared with mean DE of 46 m. Although both DE and DMNT were significantly related to TD for all plots ($n = 465$), they explained only 2 % and 8 % respectively of the variation in damage. Mean compartment DMNT varied from 36 m at Wood 5 to 611 m at McDonald, but was not significantly related to mean compartment TD. The low parrot abundance in this area, and the long flight distance between mature native trees and the McDonald plantation, probably explains the absence of damage found in this compartment.

2.3.5 Comparative effects of parrot abundance, tree growth and distance to edge on tree damage

The effect of PA on the relationship between DBH and damage can be illustrated by comparing damage in two compartments with similar tree growth. For example, in Figure 2.12 the effect of PA is shown for two plantations with similar mean DBH, and where damage clearly increased with DBH. Both plantations were from high mean annual rainfall regions, but one had moderate PA (Dunnet) while the other had high PA (Fontana). In this example, trees of 8.0 cm DBH at the Fontana plantation had almost three times the probability of damage (86 %) as those of similar size at the Dunnet plantation (30 %).



PARROT DAMAGE ASSESSMENT

Figure 2.11 Parrot damage at the Oldham 6 plantation showing distribution of damage according to edge type and distance to edge. Parrots were nesting in large *E. marginata* in the roadside vegetation on the western edge of the compartment, and in *E. wandoo* in the south-eastern corner. There were no nesting trees in the *E. rudis* remnant along the northern edge.

Statistical analysis showed there was no significant difference between the slopes of the Dunnet and Fontana regression lines, and those for Oldham 18, McKennay 2 and Wood 5, all of which had significant relationships between DBH and damage. When the DBH data from all five compartments were pooled, it was found that each compartment estimated the same population regression function ($F = 0.129$, critical $F_{(0.05)(8)(169)} \approx 1.938$) (Zar, 1996). This indicates that while damage increased with increasing PA, the nature of the relationship between DBH and damage did not change.

Similarly, the effect of DBH on the relationship between DE and damage can be shown for the Gryll and McKennay 2 plantations, both having high PA and a similar rainfall (Fig. 2.13). Mean DBH of trees at the Gryll plantations was 8.5 cm, compared with 4.8 cm at the McKennay plantation. Trees 100 m from an edge at Gryll had more than twice the probability of damage (45 %) than those at McKennay 2 (20 %). Again there was no significant difference between the slopes of the Gryll and McKennay regression lines. These examples illustrate the relationships between DBH, DE and damage, and also suggest a hierarchy of importance - PA, DBH and DE.

2.3.6 Modelling parrot damage in plantations

Variability in tree damage appears to be related to three main components:

- factors that are related to parrot abundance (i.e. mean annual rainfall, area of farmland, edge type, landscape position),
- factors related to plantation tree growth (i.e. mean annual rainfall, soil type, landscape position), and
- factors related to distance between parrots preferred habitats and plantation trees (i.e. distance to edge, distance to mature native trees, edge type).

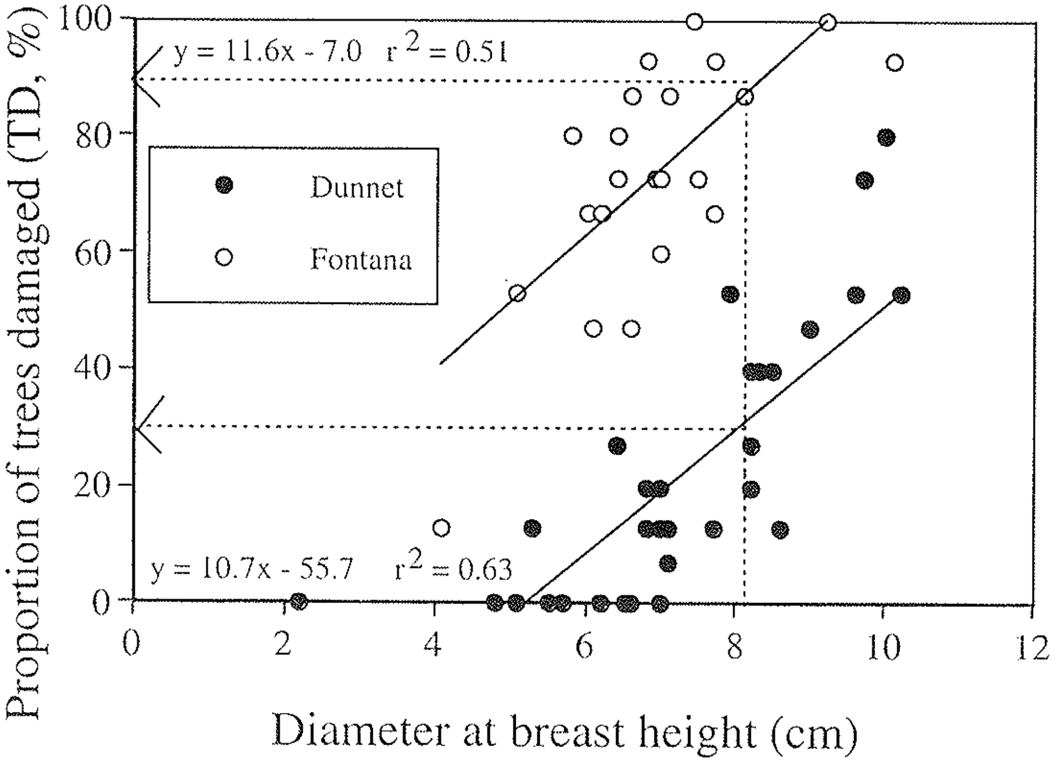


Figure 2.12. Relationship between DBH and damage for two plantations (Dunnet and Fontana) with similar mean DBH from the high rainfall region. Parrot abundance was high at Fontana and moderate at Dunnet. Predicted damage for trees with DBH of 8 cm are shown for each plantation (---).

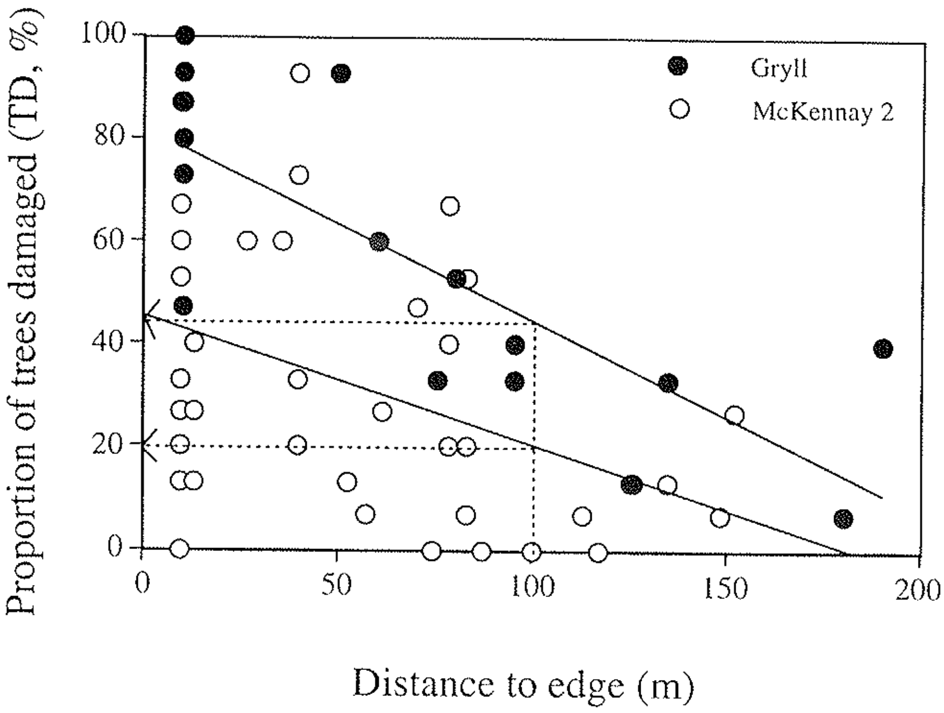


Figure 2.13. Relationships between DE (distance to edge) and damage at two plantations (Gryll and McKennay 2), with similar parrot abundance, and from the low rainfall region. Mean plot DBH at Gryll was 8.5 cm, and at McKennay 2, 4.8 cm. Predicted damage for plantation trees 100 m from an edge are shown (---).

Variables which most directly estimated these components, and which could be easily measured, were selected to construct a simple model that could be used by plantation managers to assess the risk of damage in any plantation. The number of explanatory variables used was minimised to ensure adequate replication. Variables that were correlated were avoided where possible. Backwards elimination of variables was based on rejection of variables whose partial regression coefficients (β) had t values less than the critical value ($\alpha = 0.05$).

In its simplest form (Model 1), 49 % of the variation in damage for all plots was explained using the variables of parrot abundance (PA1- low parrot abundance, PA2 – moderate parrot abundance), diameter at breast height (DBH) and distance to edge of compartment (DE) (see Methods) (Table 2.9). DBH and DE were added as covariates while PA was added as a fixed factor in the generalised linear model (GLM) using SPSS computer program (Equation 2.1).

$$\text{TD \%} = 12.3 + 7.9\ \text{DBH (cm)} - 0.2\ \text{DE (m)} - 56.8\ \text{PA 1} - 46.5\ \text{PA 2} \ldots\ldots\ldots \text{(Eqtn 2.1)}$$

When distance to nearest mature native trees (DMNT) was included as a covariate, the model explained 50 % of the variation, and when edge type (ET) (fixed factor) replaced DMNT, this increased to 54 %. The addition of ET to the model brought four interactive terms between ET and PA, as would be expected from the relationship between these variables (see Table 2.4).

When damage within each compartment was modelled separately, PA was redundant since only one assessment of PA was made per compartment. Using the three variables DBH, DE and DMNT (Model 2), up to 80 % of damage was explained (Table 2.10).

A more detailed statistical analysis of the data using S PLUS was conducted in consultation with the Mathematics Department of the University of Western Australia. Some minor changes were made to the categories before this modelling exercise, including reassessing parrot abundance for Dunnet (from

PA 2 to PA 1) and Nash 3 (from PA 1 to PA 2). The order of edge type categories was also changed from the original order (see Methods), to that in Table 2.4, in an attempt to simplify interpretation of the model output. One compartment (McDonald 5) was deleted from this analysis as no damage was recorded, presumably due to the unusually long distance between the compartment and the nearest patch of mature native trees (600 m), and the low parrot abundance generally in this area.

A new variable (PARF) was constructed to incorporate parrot abundance and rainfall since there were insufficient levels of either variable to generate an interactive term between them. Thirteen levels were chosen, ie PA 1:1005mm (Rooney), PA 1:1096 (Dunnet), PA 1:1432 (East 1), PA 2:903 (Wood 1), PA 2:1082 (Brockman), PA 2:1121 (Nash 2 and 3), PA 2:1432 (East 2), PA 3:649 (McKennay 1 and 2), PA 3:685 (Oldham 6 and 18), PA 3:796 (Gryll), PA 3:876 (Wright), PA 3:903 (Wood 5), PA 3:1165 (Fontana). Data were combined for compartments only if they had the same PA and rainfall. It was considered inappropriate to combine levels with similar PA and rainfall to reduce the number of levels, since examination of their coefficients revealed that each level had a different influence on the model. Data for tree damage was square root transformed after examination of the residuals to reduce heteroscedasticity. Curvilinear relationships were examined but did not improve the model fit.

The most appropriate linear model (Model 3) generated was of the form:

$$\begin{aligned} TD^{0.5} &= \text{intercept} + DBH + DE + DMNT + PARF + ET + DBH*PARF \\ &\quad + DBH*ET + PARF*DMNT + DE*DMNT \text{............(Eqtn 2.2)} \end{aligned}$$

The multiple r^2 value for Model 2 was 0.710, with a residual standard error of 1.932 on 392 degrees of freedom. The F statistic was 17.15 on 56 and 392 degrees of freedom, with a probability of 0.000. Coefficients for each variable and interactive terms are shown in Table 2.11.

Table 2.9
Statistical analysis of a model (Model 1) for tree damage based on the variables PA (parrot abundance), DBH (diameter of tree at breast height) and DE (distance of each plot to edge of compartment) and data from all grid plots (n = 465). See text for details of PA. The partial regression coefficient (β), standard error (SE) and significance (*P*) of each variable are shown. PA 3 is redundant in this analysis.

Variable	β	SE	*P*
Intercept	12.28	4.321	0.005
DBH (cm)	7.90	0.723	0.000
DE (m)	-0.16	0.021	0.000
PA 1	-56.84	3.679	0.000
PA 2	-46.49	2.615	0.000
PA 3	0.00	-	-

Table 2.10

Statistical analysis of multiple regressions (Model 2) using the variables DBH (diameter at breast height), DE (distance to edge), and DMNT (distance to nearest mature native trees) to predict the proportion of trees damaged (TD) for individual compartments. Significance of each regression is given (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

Plantation / compartment	n	TD (%)	r^2	*P*
McDonald	16	0	-	ns
East 1	9	1	0.120	ns
Rooney	26	6	0.245	ns
Nash 3	10	10	0.674	ns
Nash 2	15	12	0.195	ns
Brockman	25	12	0.416	**
East 2	20	17	0.444	*
Wood 1	28	21	0.380	**
Dunnet	33	21	0.626	***
Oldham 6	48	32	0.534	***
McKennay 2	42	32	0.476	***
McKennay 1	45	48	0.408	***
Wright	27	50	0.110	ns
Oldham 18	64	55	0.659	***
Gryll	17	56	0.799	***
Wood 5	18	58	0.372	ns
Fontana	22	73	0.513	**

Although all variables and interactive terms must be considered together as a model, their relative importance to the model can be evaluated to some extent by comparing the coefficients for each variable, allowing for differences in standard errors and the significance of each coefficient. Each level for PARF and ET, and their interactive terms, are compared with the last level that assumes a zero coefficient value. Thus, PARF 13 (Fontana) assumes a zero value against which all other levels of PARF are compared. Most plantations cluster around the zero value, but some are outstanding. For example, PARF 2 (Dunnet) and PARF 6 (Nash 2) have coefficient values of -7.084 and -7.350, respectively, which indicates that damage was less than expected at these plantations, whereas PARF 11 (Wright) had a coefficient of 7.711, with more damage than expected, relative to PARF 13 (Fontana). Another example is for ET, where ET 3 (scattered trees) had a coefficient of 6.033, whereas the coefficient for ET 5 (bush) was -3.768. Thus plantations adjacent to scattered trees tended to be damaged more, and those adjacent to bush damaged less, relative to ET 9 (other). A negative interaction was evident between DBH and ET3 (-0.829), indicating that damage tended to decrease with increasing DBH in plantation trees adjacent to ET 3 (scattered trees), whereas the interaction between DBH and ET 5 (bush) was positive (0.600).

The model was checked by inserting values into the equation and determining if the predicted damage was realistic. For example, assuming DBH of 8 cm, DE of 50 m and DMNT of 100m, predicted damage for PARF 11 with ET 3 was 39.2 % whereas damage for PARF 6 with ET 5 was 9.0 %. These predicted values, and others tested, appeared reasonable.

Table 2.11

Coefficients, standard errors and significance of $|t|$ for the generalised linear model (Model 3) of parrot damage using five variables (DBH, DE, DMNT, ET, and PARF) and significant interactive terms (*). See text for definition of variables.

Variable	Coefficient	SE	*P* (>$|t|$)
Intercept	0.736	4.535	0.871
DBH (cm)	1.161	0.703	0.100
DE (m)	-0.029	0.006	0.000
DMNT (m)	-0.008	0.021	0.702
PARF 1	-2.009	5.421	0.711
PARF 2	-7.084	5.041	0.161
PARF 3	-2.729	54.994	0.961
PARF 4	4.614	6.549	0.482
PARF 5	0.173	4.948	0.972
PARF 6	-7.350	5.176	0.156
PARF 7	0.517	5.427	0.924
PARF 8	-1.615	4.955	0.745
PARF 9	2.130	4.673	0.649
PARF 10	-0.838	4.669	0.858
PARF 11	7.711	6.459	0.233
PARF 12	1.615	5.091	0.751
ET 1	0.393	1.154	0.734
ET 2	0.007	1.477	0.996
ET 3	6.033	2.280	0.009
ET 4	1.229	1.996	0.538
ET 5	-3.768	1.528	0.014
ET 6	1.871	1.790	0.297
ET 7	-1.771	1.140	0.121
ET 8	-0.362	2.091	0.863
DBH*PARF 1	-0.780	0.826	0.346
DBH*PARF 2	0.421	0.763	0.581
DBH*PARF 3	5.643	8.485	0.506
DBH*PARF 4	-1.820	0.924	0.050
DBH*PARF 5	-0.400	0.760	0.599
DBH*PARF 6	0.230	0.777	0.767
DBH*PARF 7	-1.268	0.813	0.120
DBH*PARF 8	-0.343	0.739	0.643
DBH*PARF 9	-0.164	0.741	0.825
DBH*PARF 10	0.654	0.739	0.377
DBH*PARF 11	-1.015	0.872	0.245
DBH*PARF 12	-0.465	0.812	0.568
DMNT*PARF 7	0.032	0.023	0.169
DMNT*PARF 8	-0.006	0.024	0.805
DMNT*PARF 9	0.001	0.021	0.948
DMNT*PARF 10	-0.004	0.021	0.866
DMNT*PARF 11	-0.003	0.022	0.909
DMNT*PARF 12	0.018	0.022	0.417
DE*DMNT	0.000	0.000	0.020

2.4 Discussion

This survey highlighted widespread parrot damage in *E. globulus* plantations in the south-west of Western Australia. The variability in parrot damage among plantations was similar to that previously reported (Massam, 1992; Beatty and Ritson, 1993; Ritson, 1993, 1995). However, in contrast to earlier surveys, this study identified several variables that were highly correlated with parrot damage. Damage was clearly related to parrot abundance, relative tree growth, edge type, distance from compartment edge, and distance to mature native trees, but the relative importance of these variables differed between plantations. Trends between damage and other variables, e.g. soil type and landscape position were confounded by parrot abundance and tree growth, and were not included as explanatory variables in models.

Differences in parrot abundance accounted for most of the variability in damage among plantations, with damage being greater in the drier inland regions where a high proportion of the landscape has been cleared for agriculture. This coincides with areas previously reported to be at high risk of damage (Ritson, 1995), and where the abundance of *B. zonarius* has reportedly increased (Mawson and Long, 1995) due to its ability to take advantage of locally abundant supplies of cultivated grain and weed seeds.

Cereal cropping was not the only prerequisite for high parrot abundance, since damage was also severe in a plantation near Margaret River on the west coast. Parrot abundance in this region appears to be increasing, possibly in response to an increase in the area planted to wine grapes and other horticultural crops. Subsequent surveys have shown severe damage to other plantations in the Bunbury to Margaret River area (R. Quaife, pers. comm.).

Several habitat variables, which reflected local variations in parrot abundance, were related to parrot damage. Extensive damage to plantations adjacent to linear remnants of native vegetation, or to scattered native trees left in paddocks, was probably the result of greater abundance of parrots in those habitats. Differences in parrot abundance between habitats were also found in the wheat-belt of

Western Australia (Lynch and Saunders, 1991). In that study, *B. zonarius* were more common in road verges (31 % occurrence rate) than along edges of large reserves (12 %), or within reserves (8 %). This difference was attributed to the high dependence of *B. zonarius* on introduced seeds in paddocks for feeding, with linear strips of native vegetation providing easy access to paddock feed, as well as breeding and roosting sites.

Parrots tend to stay close to their nesting sites throughout the year (Forshaw, 1969; Long, 1990), although there is some movement of the local population in response to variations in food supply (Halse, 1986; Long *et al.*, 1989). This resulted in an uneven distribution of parrots within preferred habitats, which could not be adequately explained by differences in edge type alone. All tree species were used by parrots for nectar and shade, but not all species were used for nesting. The preferred species for nesting appeared to be jarrah (*Eucalyptus marginata*) and wandoo (*E. wandoo*), and nesting hollows are common in these trees. Little nesting activity was seen around marri (*Corymbia calophylla*), karri (*E. diversicolor*), flooded gum (*E. rudis*) or blackbutt (*E. patens*), although parrots nested in some very large marri in the higher rainfall regions (from Northcliffe to Margaret River). Thus, damage was often greater in plantation trees adjacent to individual mature jarrah and wandoo with nesting hollows, but no differentiation between native tree species, or between 'nesting' and 'non-nesting' mature native trees, was made during this survey.

The lower incidence of damage in plantations adjacent to forest, than to road verge or creekline vegetation, may be due to fewer large trees with suitable nesting hollows in forests. Earlier selective logging removed the largest jarrah trees, and current clearfell operations only require three 'habitat' trees per hectare to be left after logging (Inions *et al.*, 1989). Nesting hollows used by parrots were observed in large jarrah with diameters greater than 75 cm. Other estimates have suggested that suitable hollows for *B. zonarius* may develop in smaller jarrah and marri, with diameters greater than 32 cm (see Stoneman *et al.*, 1997), but no nesting activity was observed in trees of this size in the present study, indicating that they were generally unsuitable for parrots.

The assessment of parrot abundance at the plantation scale for this survey was simple but reasonably effective. A more reliable measure of parrot density may allow more robust damage-density relationships to be developed that might then be used to determine the cost effectiveness of parrot control programs. Evidence from similar studies of deer damage in the northern hemisphere suggests that damage-density relationships may differ between habitats, with the effectiveness of culling varying with the slope of the curve (Gill, 1992). A sound method for estimating parrot densities at a scale useful to plantation foresters is not yet available, mainly because of the mobility of parrots within the landscape.

Additional variables that measure the availability of alternative food resources may improve the sensitivity of the model. Ritson (1995) noted that some seasonal and spatial variations in the incidence of damage to *E. globulus* plantations could be explained by the availability of marri nectar and oat grain. *Barnardius zonarius*, however, feeds on a wide variety of food sources (Forshaw, 1969; Long, 1984) and it may prove difficult to develop an index of alternative food availability applicable to the whole south-west region.

A strong relationship between damage and distance to edge was found for some compartments in this study, which contrasts with earlier surveys of parrot damage (Ritson, 1995) where no 'edge effect' was found. Those surveys were generally conducted in small, agroforestry strip plantings to the east of the main plantation area, where parrot abundance was very high. Under such conditions, all trees were essentially 'edge' trees and were at risk of damage. Clearly, parrot damage will remain a significant problem for landholders in this drier area.

Edge effects have also been observed in pine plantations where possums severely damaged trees at the periphery of the plantation, particularly in edges adjacent to their preferred habitat (Barnett *et al.*, 1977). However, these authors noted that the damage zone increased with time. Eventually, after 16 years, trees throughout the plantation were damaged. The width of the 'edge' is therefore a function not only of animal density but also a function of time. In the present study, damage was assessed at one time only, and the width of the edge was mainly a function of parrot abundance. Total strip length per tree may thus be a better time-integrated measure of damage than simply measuring the proportion of trees damaged.

Other authors have noted a relationship between tree vigour and phloem sap feeding in arboreal marsupials that may be relevant to the present study (Craig, 1985; Kavanagh, 1987; Mackowski, 1988). Mackowski (1988) found that all trees freshly tapped for sap had high vigour, although not all vigorous trees were tapped. However, no relationship was found in that study between vigour and either DBH or tree dominance. This is not surprising since DBH does not necessarily reflect vigour, or current growth rate, particularly in forests with trees of mixed age. All plantations in the present study were two years old, so in this case, DBH was a reliable measure of growth rate since establishment.

The relationship between damage and tree growth supported the hypothesis that faster growing trees were more attractive to parrots. This is in agreement with recent 'Plant Vigour' hypotheses for plant herbivory (Coley *et al.*, 1985; Coley, 1988; Price, 1991), which suggest that trees growing in resource-rich environments should be more palatable to herbivores. However, the results of this survey show clearly that the hypotheses must be modified to include the caveat that for very mobile herbivores, distance from habitat trees is inversely related to herbivory. It was also evident that where parrot population pressure was very high (e.g. Wright plantation), all trees were vulnerable to damage. Thus the degree of selectivity is determined not only by tree attractiveness, but also by flight distance and population pressure, and may only be evident where the food resource is much larger than the demand.

Kavanagh (1987) noted that selection of trees used for sap feeding was determined more by site conditions than by species. Species of eucalypts that occupy moist, fertile sites (gullies, creeklines) were used almost exclusively by gliders, again suggesting that more vigorous trees, or those close to sources of water, were being selected for sap feeding. Although tree selection by gliders is apparently related to sap flow (Goldingay, 1987, 1991), relationships between sap flow and soil fertility, or soil water availability, have not been investigated in eucalypts.

Selection of trees by parrots in the present study was observed on several occasions. After landing on a tree, a parrot stripped a small piece of bark (about 5 cm) from the stem and appeared to taste the sap. If this was not to its liking, the parrot flew on to another tree. When a suitable tree was found, up to six parrots gathered together to strip long sections of bark from the stem. The mobility of parrots thus allowed them to sample a large number of trees and to be highly selective. Yellow-bellied gliders are less mobile than parrots but appear to have a similar method for testing the sap flow in eucalypts (Goldingay, 1987).

Parrots showed a preference for adult stems in the present study, with almost twice as many adult trees to juvenile stems being damaged in the Oldham 18 compartment. The first sign of damage in one to two-year-old plantations was frequently found on those trees that were undergoing the phase change to adult foliage. In addition, adult and juvenile trees of similar size were often observed together with consistently more damage on the adult tree, indicating that differences in damage were not due to differences in tree size alone. Previous studies have shown that stems of trees in the juvenile stage were less palatable to browsing animals, and more chemically defended, than adult stems (Bryant and Raffa, 1995).

Factors that predisposed plantations to a high risk of damage included high parrot abundance, rapid growth rate of trees, small compartment size and short distances to habitat trees. Recommendations to reduce this risk might therefore include reducing parrot abundance, reducing the use of fertilisers, designing compartments to minimise the length of edge:area ratio, and avoiding the inclusion of internal remnants of native vegetation. Reducing parrot abundance should have the greatest effect on damage, and, in many areas, parrots are currently being shot in large numbers. In 1997, one company shot over 10,000 parrots around young plantations, at a cost to the company of over $30,000 (R. Quaife, pers. comm.). This action appears to have been successful in reducing the damage, despite Bomford's (1992) earlier assertion (see pp 24) that shooting is the most ineffective method of bird control used in Australia.

Part of this success may be due to the small flock size of *B. zonarius* compared with other bird pests (Bennett, 1978; Alexander, 1990), and their apparent permanency of breeding pairs and breeding sites (Long, 1990; Crome and Shields, 1992). When parrots were removed by shooting, their breeding sites in some areas (e.g. Kloppers plantation) remained unoccupied for some time, allowing the plantation trees to escape further damage during the early growth stages. Ritson (1995) estimated that a 'Critical Period' of two years for controlling parrot damage was required to produce logs of minimum length to meet harvesting and processing criteria. Population control after this time seems to be of little economic benefit, unless parrot abundance is very high.

The relationship between damage and DBH is cause for concern for plantation managers, who aim to maximise growth rates of *E. globulus* for chip and pulpwood production. It is not feasible to recommend reducing growth rates to avoid damage, other than to avoid unnecessary use of fertiliser at establishment. It may be useful, however, to incorporate predictions from existing growth models, based on site characteristics (e.g. Battaglia and Sands, 1997; Shedley *et al.*, 1998), to determine areas that would be at high risk of damage. Shooting programs could then be made more efficient by concentrating on those areas and reducing the parrot population prior to planting. An alternative is to establish plantations in areas of low parrot abundance.

2.5 Conclusions

The results of this survey show that parrots are actively selecting *E. globulus* with fast growth rates. The basis for this selection is unknown and may be determined by physical as well as nutritional characteristics of stems. Tree selection is also determined by site factors that may override the influence of tree growth. Factors such as local variations in parrot abundance, and the distance which parrots had to fly from native habitat trees to reach plantation trees, influenced patterns of damage in plantations, and, generally, a combination of variables was required to explain variations in damage. By surveying plantations from a range of environments, with sufficient variation in damage, principle explanatory variables were identified at both compartment and regional levels. These variables were combined into a statistical model that adequately explained most of the variance in the data. To develop it further into a predictive model, information from tree growth models based on site characteristics could be incorporated. A more sensitive measure of parrot abundance within compartments would improve the reliability of the model. Plantations might then be assessed for risk of parrot damage prior to planting and appropriate action taken.

CHAPTER 3

NUTRIENT ANALYSIS OF DAMAGED AND UNDAMAGED *EUCALYPTUS GLOBULUS*

3.1 Introduction

In Chapter 2, parrot damage to *Eucalyptus globulus* was strongly related to tree growth in even-aged plantations. Although tree growth is determined by a number of soil and climatic factors, the relationship between nutrient availability and growth may be relevant to parrot damage. Field observations indicate that damage is most severe in trees growing with a plentiful supply of nutrients, particularly where sheep and cattle manure has accumulated (i.e. stock camps), and along peaty drainage lines. Furthermore, trees that show symptoms of nutrient deficiency or salinity stress are rarely damaged. The apparent preference for trees growing with abundant nutrients supports the earlier suggestion that damage may be linked to the nutritional requirements of parrots (Ritson, 1995), however the basis of this relationship is not known.

Previous studies have shown that fertilised trees are more prone to browsing damage by animals than unfertilised trees (Gessel and Orians, 1966; Andersson *et al.*, 1970; Brockley, 1988; Bryant and Raffa, 1995). Andersson *et al.* (1970) found that calcium and nitrogen fertilisers had a greater effect on damage than potassium or phosphorus fertilisers. Nitrogen is widely considered to be the major limiting nutrient for wild herbivores (White, 1978; Mattson, 1980; White, 1984; Moir, 1994; Landsberg and Cork, 1997), and has been positively related to insect herbivory in eucalypts (Fox and Macauley, 1977; Journet, 1980; Landsberg and Wylie, 1983; Ohmart *et al.*, 1985a, 1985b; Ohmart and Edwards, 1991). Gill (1992) also cited several examples of increased susceptibility of trees to deer browsing following application of nitrogen, and where soil or foliage nitrogen concentrations were increased.

Eucalypts may accumulate nutrients in stem tissues. The bark of *E. grandis*, for example, is rich in calcium, approximately four times the concentration of foliage (Turner and Lambert, 1983). In a related species, *E. saligna*, the concentration of calcium in living bark (phloem tissues) was twelve times greater than in corky outer bark (Beadle and White, 1968). This characteristic of calcium accumulation is apparently typical of smooth-barked eucalypt species (Lambert, 1981). Mean concentrations of calcium in bark of smooth-barked species (2.21 %) is up to five times greater than those in fibrous-barked species (0.42 %) (Judd *et al.*, 1995). Phosphorus accumulates in twigs, branches and stemwood of *E. globulus* and *E. diversicolor* in response to phosphorus (Dell *et al.*, 1983b; Grove, 1990; Bennett *et al.*, 1997), while nitrate accumulated in stems of *E.globulus* seedlings at high rates of nitrate supply (Shedley *et al.*, 1995). Nutrient concentrations were also significantly greater in young twigs from current season's growth than in older branches (Bell and Ward, 1984). It is not known whether such differences in nutrient concentrations can be detected by parrots or even influence their choice of stem.

Damage to *E. globulus* plantations is generally greatest in autumn and early winter (Ritson, 1995) when the quantity and quality of alternative food sources is poor. At this time, *B. zonarius* commonly feed on cereal grain gleaned from stubble and around silos. In one study, oat grain (including 'wild' oats) was present in 95 % of birds examined during winter near Wickepin in the wheatbelt, and 41 % of birds near Balingup in the central forest region (Long, 1984). Oats are rich in digestible energy (approximately 2,500 kcal kg^{-1}), but have protein concentrations well below that required for maximal growth, even for non-breeding birds. In trials conducted by Agriculture Western Australia, protein concentrations in de-husked oat grain (groats) varied from 12.6 to 16.7 %, whereas the protein requirement of parrots is approximately 20 - 25 % protein (National Research Council, 1984; Roudybush and Grau, 1986; Ullrey *et al.*, 1991). The nutritional value of groats is relevant in this context, because parrots remove the husks from seeds before they are eaten. The high intake of oat grain by parrots in the cereal growing regions may possibly predispose them to a protein deficiency.

Other nutrients required by parrots are poorly supplied in oat grain (Earle and Clarke, 1991; Ullrey *et al.*, 1991). Calcium and phosphorus are required for bone formation in young birds and for egg shell formation in breeding birds, and a dietary intake for psittacines of 1.1 % calcium and 0.8 % phosphorus has been recommended (Ullrey *et al.*, 1991). The concentration of these elements in locally grown groats varied from 0.04 to 0.08 % for calcium and 0.36 to 0.51 % for phosphorus (G. Crosby, Agriculture Western Australia, unpublished data), similar to values found elsewhere (0.04 % and 0.34 %, respectively) (Earle and Clarke, 1991). Many seeds have a large phosphorus to calcium ratio (National Research Council, 1984), so that supplementation of calcium is often required for healthy growth of birds fed a seed-only diet. It is therefore possible that parrots may be seeking a calcium supplement from the inner bark of *E. globulus*. Other common nutrient deficiencies in de-husked seeds include sodium, manganese, zinc, iron, iodine, selenium, vitamins A, D, E and K, lysine, riboflavin, pantothenic acid, niacin, vitamin B-12 and choline (Ullrey *et al.*, 1991).

In this chapter, stem and leaf samples of *E. globulus* were analysed to determine whether parrot damage was related to the nutrient status of trees. Two preliminary trials were designed to test this hypothesis. In the first trial, the nutrient status of paired plots of damaged and undamaged trees were compared from two-year-old plantations. The nutrient status of adult and juvenile trees was also compared in view of the apparent preference shown by parrots for adult stems in Chapter 2. In a second trial, transects of trees with a range of parrot damage were analysed in a compartment where damage was previously found to be primarily related to the rate of tree growth (Chapter 2).

3.2 Methods

3.2.1 Paired plots trial

Design: Six paired plots of 15 trees each (three rows x five trees) were selected from two plantations of *E. globulus* previously surveyed for parrot damage (Chapter 2). The two-year-old plantations, Oldham and McKennay, in the low mean annual rainfall region (685 and 649 mm, respectively), had similar amounts of parrot damage and high parrot abundance. Each pair comprised one 'heavy damage' plot (> 25 cm mean strip length and > 75 % trees damaged) and one 'light damage' plot (<10 cm mean strip length and <15 % trees damaged). Mean tree damage for heavy damage plots was 85.5 %, and for light damage plots, was 4.3 %. Plots in each pair were less than 100 m apart to reduce the effects of habitat and local parrot abundance on the level of damage.

Sampling: Plots were marked and trees re-measured for diameter at breast height (DBH) in October 1995, three months after the initial survey of parrot damage (Chapter 2). From one way ANOVAs, there was no significant difference between mean DBH of trees within heavy damage plots to that of light damage plots, at either measurement time, and there was no significant difference in mean growth rate over this time. The intention of using similar sized trees was to reduce the possible influence of physical factors (e.g. tree height and internode length) that may affect tree attractiveness to parrots, so that differences in damage due to nutrient status alone could be determined. Increases in stem diameter were apparently unaffected by the high rates of damage (Table 3.1).

Soil was sampled by taking two core samples per plot, at 0 - 10 cm and 10 - 30 cm soil depth, with a 10 cm diameter soil auger, from the undisturbed soil between tree rows.

Table 3.1
Mean diameter at breast height (DBH) of six paired plots measured during the survey of parrot damage in July, 1996 (DBH_1) and in October, 1996 (DBH_2). The absolute growth rate (AGR) of trees over this time (DBH_2 - DBH_1/T_2 - T_1) is also shown. Mean values are shown for plots with either 'heavy' damage (HD) or 'light 'damage (LD) (see Methods).

Paired Plots	DBH_1 cm		DBH_2 cm		AGR mm day^{-1}	
	HD	LD	HD	LD	HD	LD
1	5.4	4.6	6.6	6.4	0.101	0.152
2	7.0	5.4	8.6	6.6	0.134	0.101
3	5.5	5.2	6.6	6.4	0.097	0.107
4	6.8	4.9	8.1	6.2	0.120	0.119
5	4.2	5.4	5.1	6.5	0.083	0.102
6	6.3	5.1	7.6	5.9	0.121	0.075
Mean	5.9	5.1	7.1	6.3	0.109	0.109
SE	0.4	0.1	0.5	0.1	0.008	0.010

Pairs of youngest fully expanded leaves (YFEL) were sampled from branches cut from upper canopy using a tree pruner. Six adult and six juvenile undamaged branches were cut per plot from dominant or co-dominant trees. Adult branches were taken from trees that had at least the upper third of the canopy with adult leaves, while juvenile branches were from trees in each plot that had no adult leaves present. YFEL leaf pairs were separated evenly before bulking. Half of the YFELs were placed in paper bags for oven drying for two days at 70° C for nutrient analysis. Discs were cut from the other YFELs in the field with an 8 mm diameter corer and placed in pre-weighed plastic vials, sealed and kept on ice until transported to the laboratory. The vials were re-weighed to determine fresh weight (FW). Discs were then oven dried for two days at 70° C to determine dry weight (DW). One sample each of juvenile YFELs and juvenile mature leaf blades (MLB, i.e. YFEL + 5) were also taken from the mid-crown position for nutrient analysis only. Approximately 40 leaves from each plot were bulked for analysis.

Internodal sections of stem were cut from the same adult and juvenile branches taken from the upper crown for leaf sampling. Six to ten stem sections per plot, each 1.5 - 2.0 cm diameter, with bark attached, were bulked and placed in paper bags. Small sections approximately 0.5 - 1.0 cm long were cut from these stems and placed in pre-weighed vials, as before, for FW:DW analysis.

Nutrient analysis: Approximately 0.1 g of oven-dried plant material (leaves and stems) was accurately weighed and digested in 3 ml conc. H_2SO_4 with salicylic acid and H_2O_2 added for total nitrogen analysis (Fleck, 1974; Dalal *et al.*, 1984). Total nitrogen was determined on the digest colorimetrically using a modified Berthelot reaction (Searle, 1984). Phosphorus and potassium were also determined in this digest for a number of samples as a crosscheck with ICP analysis (see below). Phosphorus was determined colorimetrically using ammonium molybdate, potassium antimony tartrate and ascorbic acid (after Murphy and Riley, 1962), while potassium was determined using atomic absorption spectrometry (AAS) with caesium as an ionisation suppressant. All analyses were duplicated and standard reference materials and blanks were taken through the digestions and subsequent analyses.

A subsample of each dried sample was finely ground and re-dried, then analysed for total nutrient content. Approximately 0.5 - 1.0 g of oven-dried plant material was accurately weighed into test tubes and 10 ml of conc. HNO_3 added. Test tubes were placed in an aluminium digestion block and pre-digested at room temperature overnight, then digested at 147° C for 600 minutes. When completed, the digest was diluted with 25 ml 1 % (v/v) nitric acid, decanted and stored for total element analysis (P, S, Ca, K, Mg, Mn, Zn and Cu) by inductively coupled plasma spectrometry (ICP) (Zarcinas *et al.*, 1987).

Soil samples were air-dried, sieved (2 mm) and sub-sampled for analysis. pH and electrical conductivity (EC) were determined in a 1:5 soil:water solution. Phosphorus and potassium were extracted for 16 h at 25° C in 1:100 soil:solution of 0.5 M $NaHCO_3$ (after Colwell, 1963), and analysed colorimetrically (see above). Organic carbon (OC) was determined by digesting 1 g soil in hot dichromate-sulphuric acid mixture (Walkley and Black) followed by potassium iodide - sodium thiosulphate titration using Iotet for endpoint determination (P. Longbottom, formerly Aquanal Services, pers. comm.).

<u>Data analysis</u>: Mean nutrient concentrations, DBH and FW:DW were compared for heavy and light damage plots, and for adult and juvenile leaves and stems, using one-way ANOVA. Regression analysis was used to determine the strength of relationships between corresponding leaf and stem nutrient concentrations.

3.2.2 Transect trial

<u>Design</u>: Twenty four plots (four transects x six plots) were selected from a compartment (Oldham 18) which had been surveyed for parrot damage six months earlier (see Chapter 2). Damage in these selected plots had varied from zero to 93 % trees damaged at the time of surveying, while mean plot DBH

ranged from 2.2 - 7.5 cm. In this compartment, damage was strongly related to DBH with no effect of distance to edge (see Table 2.8).

Sampling: Each plot of 15 trees (three rows x five trees) was marked and measured for DBH and height during January 1996. Parrot damage was re-assessed by estimating the total length of bark 'recently stripped' (i.e. no callus on wounds) on the central stem of each plot tree. Soil samples were collected (0 - 10 cm) from undisturbed soil between tree rows. A bulk sample of approximately 40 leaves (YFELs) was collected from a sub-sample of twelve plots (2 transects x 6 plots) by cutting six adult branches per plot from the upper canopy.

Nutrient analysis: Soil samples were air-dried and sieved as in the previous trial. Total nitrogen, as an index of soil fertility, was analysed by combusting the soil at 950° C in oxygen using a LECO FP-428 nitrogen analyser. YFELs were oven-dried and finely ground as before, and digested in H_2SO_4-H_2O_2 for total N, P and K analysis. Total N and P were determined colorimetrically, while total K was determined by AAS. Samples were analysed in duplicate with standard reference material and blanks included in each digestion batch.

3.3 Results

3.3.1 Paired plots trial

Stem analysis: There was little difference in the mineral composition of stems from plots with heavy or light parrot damage (Table 3.2a). Heavy damage plots (adult and juvenile stems combined) had lower concentrations of magnesium (0.11 %) than light damage plots (0.14 %). Concentrations of calcium and manganese were less in heavy damage plots but the difference was not significant ($P = 0.07$). Concentrations of calcium (0.39 %) and copper (4 $\mu g\ g^{-1}$) were significantly less in adult stems (all plots combined) than juvenile stems (0.50 % and 5 $\mu g\ g^{-1}$, respectively) (Table 3.2a). No differences were found between heavy and light damage juvenile stems, and only magnesium differed between heavy and light damage adult stems (0.09 % and 0.14 %, respectively).

Leaf analysis: Potassium concentrations in YFELs (adult and juvenile combined) sampled from heavy damage plots were less (0.47 %) than in light damage plots (0.55 %) (Table 3.2b), but otherwise the mineral composition of foliage sampled from damaged and undamaged trees was similar. Adult YFELs from all plots had a greater concentration of nitrogen than juvenile YFELs (1.78 % and 1.56 %, respectively). When juvenile YFELs were considered separately, the concentration of zinc was greater in heavy damage plots (22 $\mu g\ g^{-1}$) than in light damage plots (18 $\mu g\ g^{-1}$), whereas for adult YFELs, the concentration of potassium was less in heavy damage plots (0.47 %) than in light damage plots (0.58 %).

Leaf versus stem nutrient status: From regression analysis, there was a positive relationship ($P<0.05$) between the nutrient status of adult YFELs and stems for P, K, S, Mn and Cu in the twelve plots sampled. Linear relationships fitted the data better than curvilinear ones for all those elements except Mn (Fig. 3.1).

Mid-crown leaf analysis: In mature leaf blades (MLBs) sampled from the mid-crown position, concentrations of nitrogen were greater, and that of calcium less in heavy damage plots than in light damage plots (Table 3.3). The concentrations of all elements differed significantly between mid-crown YFELs and MLBs for HD and LD plots combined, with N, P, S, K, Zn and Cu being greater in YFELs than in MLBs, while Mg, Ca and Mn were less.

Water content: There were no significant differences between heavy and light damage plots, or between adult and juvenile trees, in the mean FW:DW ratio of stems or leaves (data not presented). The FW:DW varied from 2.0 to 2.7 for adult leaves and 2.3 to 2.6 for juvenile leaves. The ratios for adult and juvenile stems varied from 2.4 to 3.0.

Table 3.2.

Comparison of the nutrient composition of (a) young stems and (b) youngest fully expanded leaves (YFELs) of *E. globulus*, sampled from plots with either 'heavy' (HD) or 'light' (LD) parrot damage (n = 12). Mean concentrations for adult and juvenile stems (a) and YFELs (b) from all plots are also shown. Significant differences between mean concentrations for each element are indicated (**P*<0.05, ***P*<0.01, ****P*<0.001).

Nutrient	HD	LD	*P*	Adult	Juvenile	*P*
(a)						
N %	0.62	0.62		0.62	0.62	
P %	0.09	0.10		0.10	0.10	
K %	0.55	0.60		0.63	0.52	
S %	0.05	0.05		0.05	0.05	
Ca %	0.41	0.48		0.39	0.50	***
Mg %	0.11	0.14	**	0.11	0.14	
Mn μg g^{-1}	53	86		72	70	
Zn μg g^{-1}	10	10		10	10	
Cu μg g^{-1}	4	4		4	5	*
(b)						
N %	1.64	1.70		1.78	1.56	**
P %	0.15	0.16		0.16	0.15	
K %	0.47	0.55	*	0.52	0.50	
S %	0.15	0.14		0.15	0.14	
Ca %	0.62	0.62		0.60	0.64	
Mg %	0.18	0.16		0.17	0.18	
Mn μg g^{-1}	122	163		145	141	
Zn μg g^{-1}	19	20		20	20	
Cu μg g^{-1}	6	7		7	6	

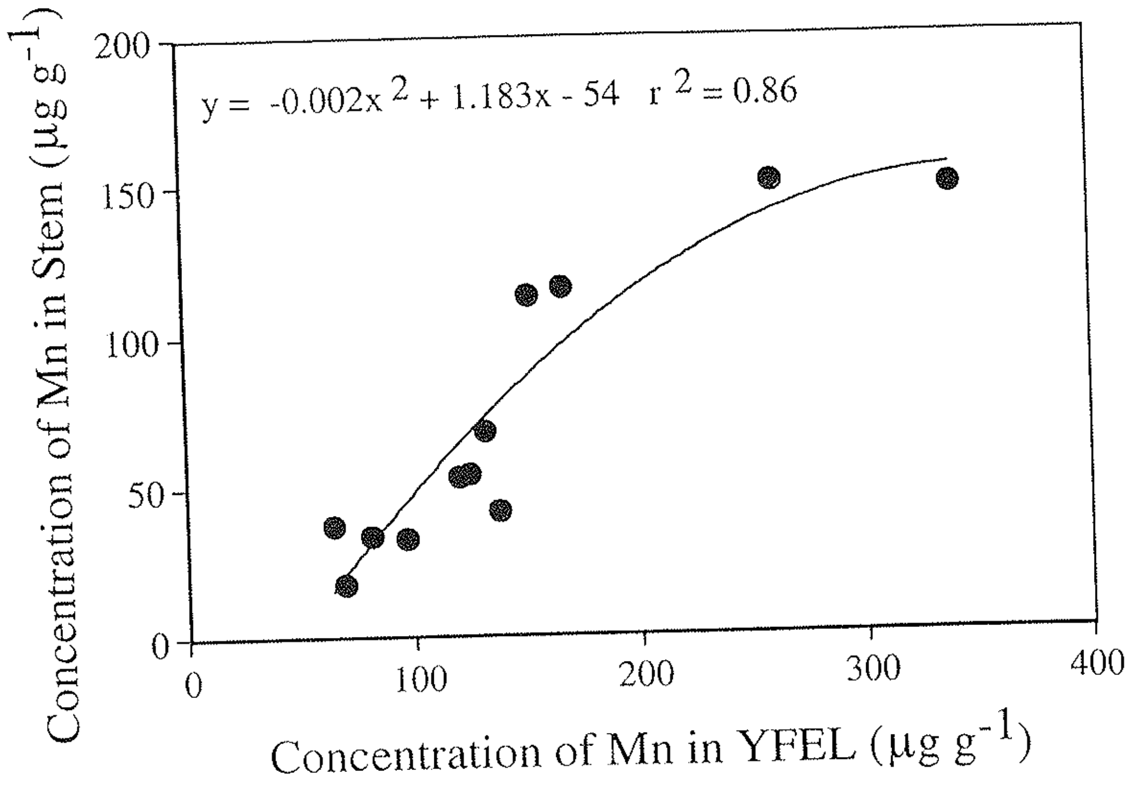


Figure 3.1 Quadratic relationship between Mn concentration in youngest fully expanded leaves (YFEL) and in stems of adult trees from all plots sampled in the paired plot study (n = 12).

Table 3.3

Concentrations of nutrients in mid-crown mature leaf blades (MLBs) of *E. globulus* sampled from plots with either 'heavy' (HD) or 'light' (LD) parrot damage (n = 12). A comparison between young fully expanded leaves (YFELs) and MLBs from the mid-crown for all plots is also shown. Significant differences between mean values for each nutrient are indicated (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

Nutrient	HD	LD	*P*	YFEL	MLB	*P*
N %	1.38	1.21	*	1.50	1.30	**
P %	0.09	0.10		0.15	0.09	***
K %	0.41	0.42		0.50	0.42	***
S %	0.13	0.12		0.14	0.12	***
Ca %	1.26	1.57	**	0.64	1.41	***
Mg %	0.24	0.27		0.18	0.25	***
Mn μg g^{-1}	207	398		141	302	*
Zn μg g^{-1}	8	7		20	8	***
Cu μg g^{-1}	4	3		6	4	***

Table 3.4

Analysis of soil samples taken from plots of *E. globulus* (n = 6) with either 'heavy' (HD) or 'light' (LD) parrot damage (see Methods). Mean values for both topsoil (0 - 10 cm) and subsoil (10 - 30 cm) samples are shown (na = not analysed).

	Topsoil		Subsoil	
Soil test	HD	LD	HD	LD
pH	5.5	5.1	5.6	5.3
EC mS m^{-1}	19	16	10	8
P μg g^{-1}	71	42	31	17
K μg g^{-1}	147	112	95	66
OC %	5.6	4.3	na	na

Soil analysis: There were no significant differences in soil characteristics between plots with heavy or light parrot damage (Table 3.4). Mean values for extractable P and K tended to be greater for heavy damage plots but due to the high variability between plots, this difference was not significant. Although standard deficiency ranges for soil nutrients have not been determined, none of the soils tested would be considered to have inadequate concentrations of P or K for tree growth. There was no relationship between soil and leaf nutrient concentrations for either P or K for the twelve plots sampled.

3.3.2 Transect trial

Stem volume: Stem volumes (V) were estimated using the conic function V = 1/3 (BA*HT), where BA is the basal area [π*(DBH/2)^2] at breast height. Parrot damage was positively related to stem volume in transect plots (n = 24) across the Oldham 18 compartment ($r^2 = 0.415$, $P<0.001$). This was expected given the strong relationship between damage and DBH found in this compartment in the earlier survey of parrot damage (see Chapter 2). Tree height was strongly related to DBH ($r^2 = 0.897$, $P<0.001$) for these plots (Fig. 3.2), confirming the use of DBH as a reliable measure of tree growth for young *E. globulus*. Stem volume was unrelated to soil total N (n = 24), due to the high concentrations of total N in all samples (0.38 - 0.73 %), and consequently, there was no relationship between soil N and parrot damage.

Leaf analysis: Stem volume was negatively related to foliar P ($r^2 = 0.344$, $P<0.05$) (Fig. 3.3), and positively related to foliar K ($r^2 = 0.340$, $P<0.05$) (Fig. 3.4), but there was no significant relationship between volume and foliar N. Damage was also negatively related to foliar P ($r^2 = 0.381$, $P<0.05$), and tended to increase with foliar K ($P = 0.08$).

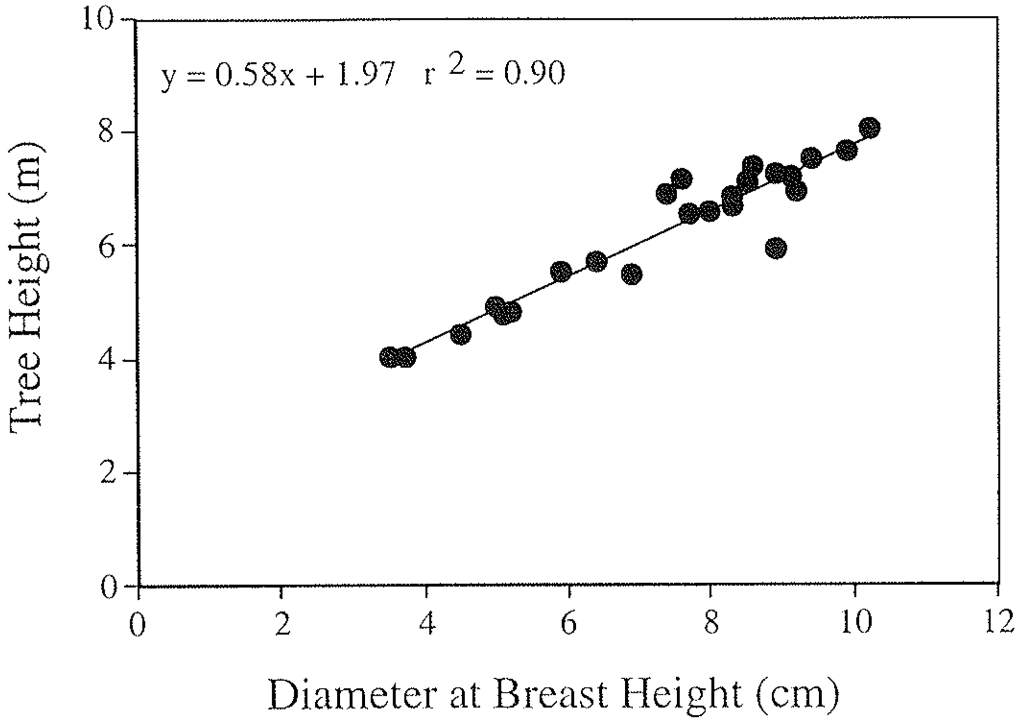


Figure 3.2 Relationship between mean diameter at breast height (DBH) and mean tree height (HT) for 24 transect plots in the Oldham 18 plantation.

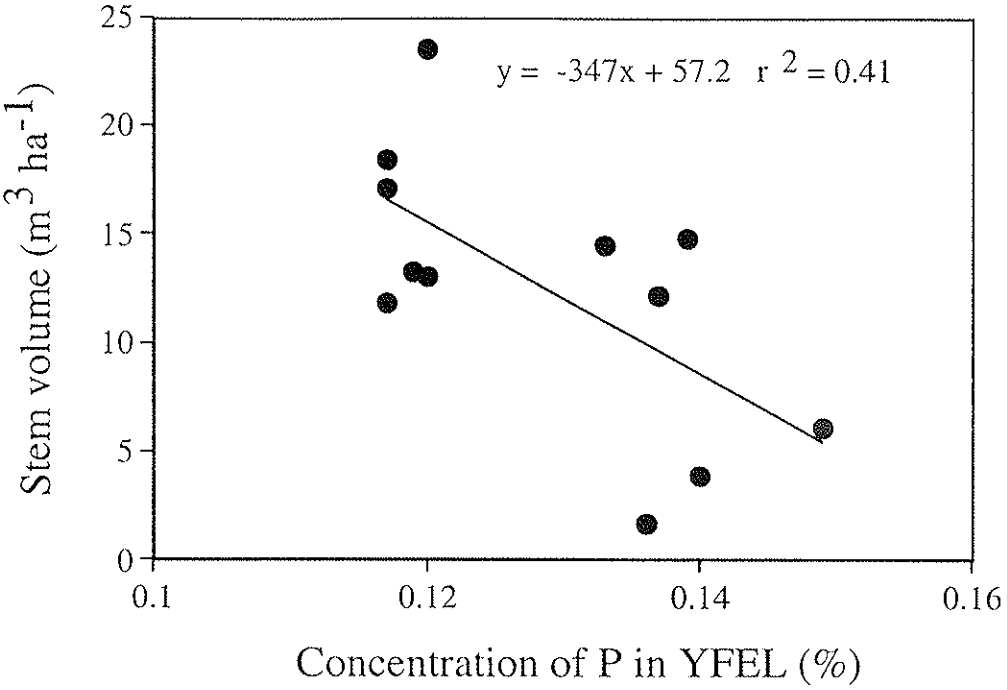


Figure 3.3 Negative relationship between foliar P concentration and stem volume of *E. globulus* for 12 transect plots in the Oldham 18 plantation.

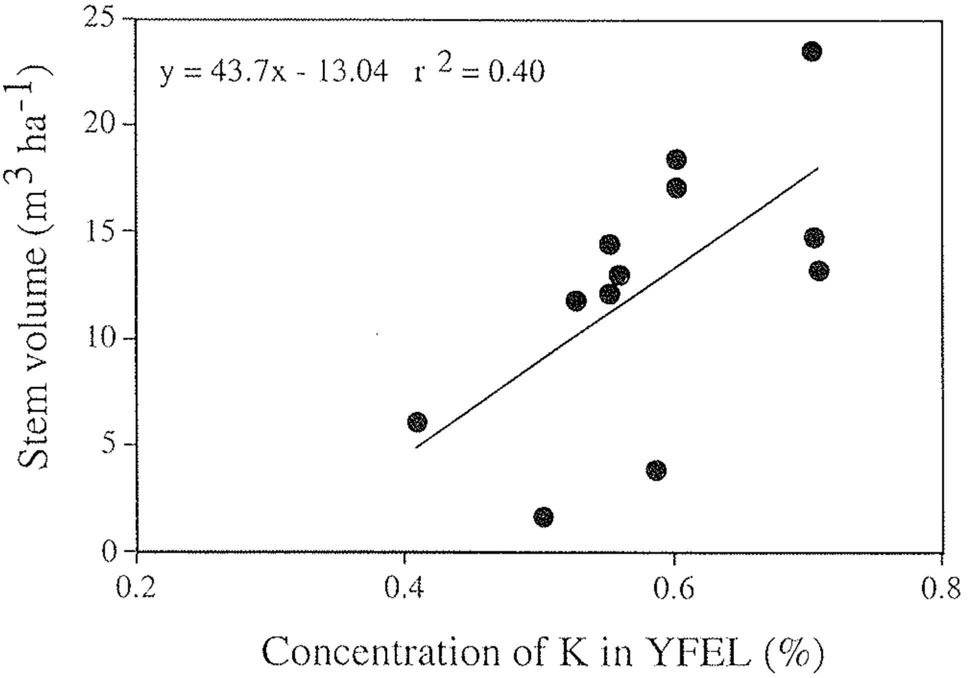


Figure 3.4 Positive relationship between foliar K concentration and stem volume of *E. globulus* for 12 transect plots in the Oldham 18 plantation.

3.4 Discussion

The data from these preliminary trials suggest that nutrient status of *E. globulus* was not closely related to parrot damage. In none of the plots were concentrations of nutrients unusually large. The preference of parrots for faster growing trees shown previously cannot simply be explained on the basis of increased nutrient concentrations in preferred trees. Parrots presumably gain some nutritional benefit from the cambium and phloem sap, but factors other than total nutrient concentrations in stems and foliage appear to determine tree selection. This is in general agreement with other studies, which generally fail to demonstrate simple relationships between nutrient content of bark, and frequency of bark stripping, even though bark was evidently used as food (Gill, 1992).

There was some evidence that concentrations of potassium may be related to parrot damage, but the results were ambiguous. In the transect trial in Oldham 18, where damage increased with stem volume, damage and foliar potassium concentrations were seemingly related. However, in the paired plots trial, where trees of similar size were compared, potassium concentrations in trees which were heavily damaged was significantly less than in trees which were lightly damaged by parrots. Two possible explanations are:

a) damage may be related to potassium concentration, but in the paired plots trial, some other factor, such as distance to edge or edge type, masked the relationship.

b) the relationship may have been coincidental rather than causative, with damage being related primarily to tree size, regardless of potassium concentration. If so, then any factor, either nutritional or physiological, which limited tree growth could be related to damage.

In the paired plots trial, it was assumed that all plots selected had equivalent opportunity for damage by parrots. However, several plots in the light damage group remained undamaged, and it cannot be determined whether this was because the trees were unattractive to parrots, or because the parrots had not yet found them. Undamaged trees with high nutrient concentrations would tend to mask underlying trends between nutrient concentration and damage. This effect would be more pronounced in compartments in which distance to edge or edge type, rather than DBH, strongly influenced the amount of damage. In this trial, mean distance to edge for plots with light damage was 99 m compared with only 17 m for heavily damaged plots. The magnitude of these interacting factors was difficult to assess when selecting plots, and although an attempt was made to control for these factors, they may have influenced the results to some extent. There was no evidence that distance to edge had any effect on nutrient concentrations.

Specific appetites for calcium, zinc and phosphorus in domestic birds are known (Hughes, 1979), but potassium concentration by itself is unlikely to be a primary determinant of tree selection. It may be an indicator of other physical or physiological factors to which parrots respond. For example, potassium deficiency is known to reduce translocation of photosynthates from source leaves causing a reduction in sugar concentration in stems and phloem sap in other plants (Hartt, 1969; Doman and Geiger, 1979; Cakmak *et al.*, 1994). It is unlikely that the relatively small differences in potassium concentrations found in this trial would cause differences in sugar concentrations large enough to be detected by parrots.

Phosphorus concentrations in leaves were also related to stem volume at Oldham 18, but this relationship was negative. This response is not uncommon in eucalypts, and may be due to nutrient interactions (especially N x P) or 'dilution of nutrients' with increased growth (Lamb, 1976, 1977; Schönau and Herbert, 1982; Lambert, 1984). Thus smaller, less damaged trees tended to have greater phosphorus concentrations in the leaves.

In the Dunnet and Fontana plantations (Chapter 2), where damage was strongly related to DBH, a variety of factors determined tree growth. Soil and foliar analysis of the Dunnet plantation by Bunnings Treefarms indicated that nitrogen, copper and zinc, as well as potassium deficiencies were associated with poor growth (C. Shedley, pers. comm.). In addition, the presence of hard lateritic caprock at the Fontana plantation appeared to limit root growth and access to soil water. Soil water storage capacity and salinity are important factors in determining growth of *E. globulus* in Western Australia (Battaglia and Sands, 1997; Hingston *et al.*, 1998; Shedley *et al.*, 1998), suggesting that the relationship between damage and growth rate may not be associated with specific nutrient effects.

Nitrogen concentration at the mid-crown position was related to damage in the paired plots trial, but no relationship was found between damage and nitrogen in the upper crown position for either trial. In these trials, nitrogen concentration did not vary greatly and nitrogen did not appear to be limiting growth. In another study (Andersson *et al.*, 1970), where nitrogen was limiting growth, bird damage was greater in trees fertilised with nitrogen for ten years, than in trees given other fertilisers, but again this appeared to be related more to growth responses than to foliar nitrogen concentrations. In that study, the relative basal area of plots receiving nitrogen was more than double that of control plots or plots receiving non-nitrogenous fertilisers.

Adult leaves from all plots had greater concentrations of nitrogen than juvenile leaves, but there were no differences in nitrogen concentrations of stems. Calcium concentrations were less in adult stems than juvenile stems, so the greater level of damage to adult stems was evidently not based on parrots seeking a calcium supplement, as previously suggested. In Chapter 2, adult stems were damaged more severely than juvenile stems, but this may have been influenced by the fact that trees that had undergone the phase change were larger and more conspicuous to parrots. In the present trial, trees of similar size were compared, and there were no obvious differences between adult and juvenile stems for the nutrients measured. It is possible that juvenile stems are better chemically defended against browser than adult stems, as has been shown in other woody plant species (Bryant and Raffa, 1995). The apparent preference for adult stems of *E. globulus* was investigated further in aviary trials.

In these preliminary trials, recently caught parrots were kept in two large aviaries and offered stems (1 m long x 1 - 2 cm diameter, with leaves removed) attached in an upright position to the side walls of the aviary. Adult and juvenile stems were collected each morning from the upper canopy of young even-sized *E. globulus* on four consecutive days in each of two weeks, and left in the aviaries for two hours per day. On each day, the mean length of bark stripped from adult stems was greater than from juvenile stems, whether stems were randomly placed or grouped according to phase. Thus, the preference for adult stems observed in plantations was repeated by parrots in captivity, where neither tree height nor leaf shape could influence their choice. [These preliminary trials were conducted under license from the Department of Conservation and Land Management (License No. SF002050), and with approval from the Animal Experimentation Ethics Committee of the University of Western Australia (Approval No. 183/97)].

Little is known about differences in physical characteristics or chemical composition of eucalypts, but there are a number of factors that may affect stem palatability and attractiveness to parrots. For example, the waxy bloom that covers juvenile bark readily adheres to skin and may interfere with normal preening of feathers. On the other hand, adult stems are shiny with variable amounts of dark red anthocyanin pigmentation, similar to that of semi-mature fruit (gumnuts) of *Corymbia calophylla* sought by parrots in winter. In addition, juvenile leaves are sessile, and tend to cover the stem, whereas the petiolated adult leaves hang away from the stem, exposing a greater length of stem to parrots. Chemical composition of the bark itself may have little influence on stem choice, since parrots do not chew or ingest the bark (see Fig. 1.4 and description of bark stripping on pp 7). I found no evidence of bark fragments in any of the crop contents examined from a random sample of over 260 parrots shot in plantations of *E. globulus* during 1996. However, it is possible that parrots may be influenced by the concentration of chemical defence compounds in cambial tissues on which they feed (e.g. soluble phenolic compounds).

The apparent preference for adult stems may be of practical interest, since height to phase change in *E. globulus* is a heritable trait (Dutkowski and Potts, 1998) and could be included in breeding programs. For example, the North-eastern Tasmania race of *E. globulus* in particular is characterised by persistent juvenile foliage, whereas other races, such as Wilsons Promontory Lighthouse race, have early transition to adult foliage. However, initial assessment of old parrot damage in local provenance trials indicated that damage was primarily related to DBH, with no effect of provenance (G. Dutkowski, pers. comm.). Screenings of different provenances for parrot damage in the first two years of growth may provide further insights into the basis of stem selection by parrots, but the results need to be carefully evaluated.

3.5 Conclusions

There were no apparent relationships between parrot damage and tree nutrient status in these trials. Only minor variations in soil, foliar and stem nutrient concentrations were found between plots, despite large differences in both damage and tree growth. Foliar analysis alone provided little indication of trees that were prone to damage. External factors, such as distance to edge or edge type, may have masked some relationships between damage and tree nutrient status, but such interference was probably minor. Differences in damage may be related to physiological or morphological differences in trees associated with growth responses rather than to specific nutritional effects.

CHAPTER 4

SPATIAL, TEMPORAL AND GROWTH-RELATED VARIATIONS IN YIELD AND CHEMICAL COMPOSITION OF PHLOEM SAP OF YOUNG *EUCALYPTUS GLOBULUS*: IMPLICATIONS FOR SAP-FEEDING ANIMALS

4.1 Introduction

There are remarkable similarities between the sap-feeding behavior of Twenty-eight parrots (*Barnardius zonarius*) in plantations of *Eucalyptus globulus* in the south-west of Western Australia (e.g. Ritson, 1995), and that of the south island kaka (*Nestor meridionalis*) in New Zealand (O'Donnell and Dilks, 1989) and some Australian arboreal marsupials (Smith, 1982; Smith and Russell, 1982; Henry and Craig, 1984; Goldingay, 1987, 1991). Phloem sap feeding by these animals in temperate eastern Australia is seasonal, and is thought to help overcome periodic shortages in other high-energy foods, such as nectar, lerp, manna and insects. However, seasonal variations in sap feeding may also reflect differences in sap flow and sap content, with greatest use of this food resource when it is readily obtained and most nutritious. Given the dietary importance of phloem-derived materials to many of these animals, it is surprising that so little attention has been given to the study of seasonal and spatial variations in phloem sap composition of their host trees.

Information on phloem sap composition for eucalypts has been limited. Zimmermann and Ziegler (1975) qualitatively assessed the composition of sugars in four eucalypt species (i.e. *E. globulus*, *E. viminalis*, *E. saligna* and *E. naudiniana*) and found raffinose-type sugars in appreciable amounts as well as sucrose. Recent studies of sap feeding by gliders have shown that total sugar concentrations in phloem-derived exudates varied from 18.0 to 37.1 % (w/v) sucrose equivalents (Henry and Craig, 1984; Goldingay, 1987), but the cause of this variation was not determined. In other plant species, sucrose concentrations in phloem sap generally increased with water stress (Hall and Milburn, 1973) and decreased in response to potassium and magnesium deficiency (Doman and Geiger, 1979; Cakmak *et al.*, 1994).

A method for sampling phloem sap from small incisions made in the bark of two- and three-year-old *E. globulus* has been recently described using data from this current study and other related studies (Pate and Arthur, 1998; Pate *et al.*, 1998). This 'bleeding' technique has been used widely with herbaceous species, notably *Lupinus albus*, (e.g. Pate *et al.*, 1974) and *Ricinus communis* (e.g. Smith and Milburn, 1980), but less commonly with woody species (e.g. Zimmermann and Ziegler; 1975; Hocking, 1980). Other techniques for collecting phloem sap (e.g. aphid stylet exudates, exudates from cut fruit pods, petioles, and inflorescences, and promotion of exudation following treatment with chelating agents such as EDTA) are well documented (e.g. Fisher and Frame, 1984; Pate *et al.*, 1985; Geigenberger *et al.*, 1993; Cakmak *et al.*, 1994; Girousse *et al.*, 1996) but have not been applied to eucalypts.

Chapter 2 described a survey of tree damage that resulted from the sap-feeding behaviour of twenty-eight parrots (*Barnardius zonarius*) in even-aged plantations of *E. globulus*. Although damage was strongly influenced by the abundance of parrots, tree growth was an important factor, with parrots preferentially selecting faster growing trees. The incidence of damage is also known to fluctuate on a seasonal basis, being greatest during autumn and early winter, with very little damage recorded during spring (Ritson, 1995). I therefore hypothesised that both seasonal and spatial patterns of parrot damage might be associated with differences in phloem sap composition, which, in turn, might depend on prevailing growing conditions.

The aims of this study were to (i) further develop the methodology for phloem sap collection from young, even-aged *E. globulus*, (ii) examine the variability in phloem composition of *E. globulus* within stems, between spring and autumn, and among a wide range of sites, and iii) relate differences in phloem composition to growth of *E. globulus*.

4.2 Methods

4.2.1 Site selection

Plantation sites were chosen from the wide range of climatic and edaphic conditions where *E. globulus* is grown commercially in the southwest of Western Australia, and where parrot damage has been observed. This region has a pronounced Mediterranean climate of hot summers with little rainfall between December and April, and cool wet winters.

Sixteen plots of 10 trees each (two rows x five trees) were selected from three adjoining 16-month-old plantations of *E. globulus*, in November 1995 (i.e. Klopper, Nield and AEL, collectively referred to in this study as the Klopper plantation; K1 - K16; E 438000 m, N 6249000 m), approximately 20 km east of Bridgetown, Western Australia (Fig. 4.1). An additional plot, which was being severely damaged by parrots, was selected in April, 1996 (K17). A further six plots were selected in the East plantation (E1 - E6; E 406000 m, N 6170000 m), approximately 12 km west of Northcliffe, and six plots from the Naranbee plantation (N1 - N6; E 474000 m, N 6217000 m), approximately 60 km south-east of Bridgetown (E 420130 m, N 6242439 m). Mean annual rainfall for the three plantations, taken from nearby rainfall stations, were Klopper 663 mm, Naranbee 696 mm and East 1432 mm. The plots were chosen to represent the range of soil types at each plantation and were expected to produce different rates of tree growth. All plots were located within 100 m of known parrot activity, and within 100 m of the edge of a compartment within a plantation.

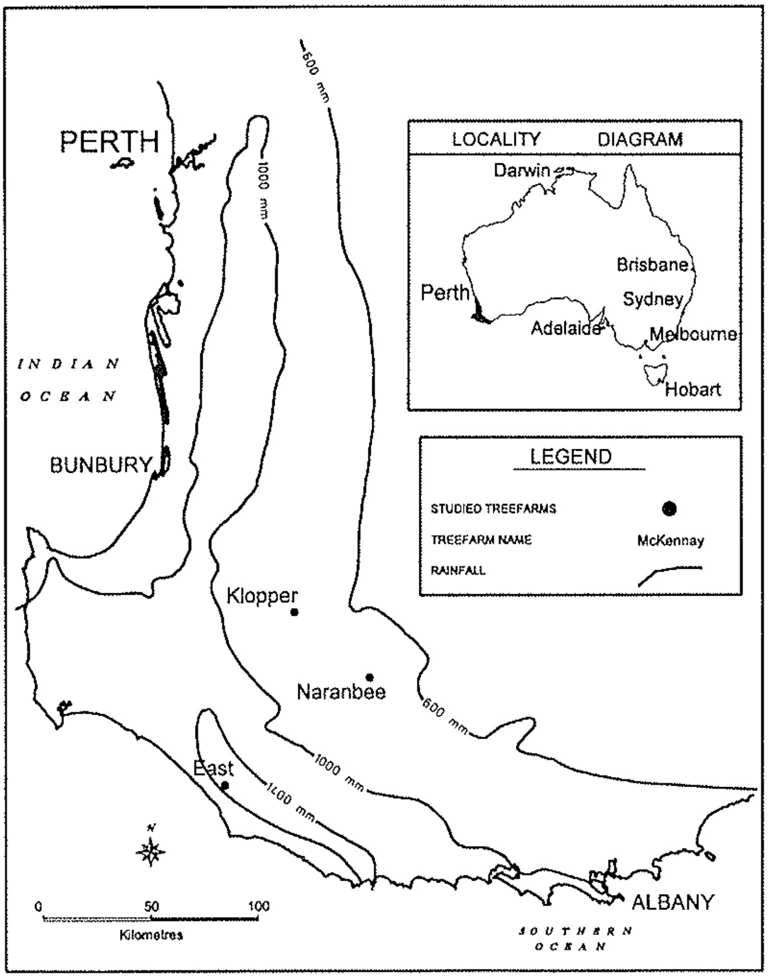


Figure 4.1 Map showing location of the Klopper, East and Naranbee plantations planted in 1994. The East plantation is in the high rainfall region (1200 – 1400 mm), and the Klopper and Naranbee plantations are in the low rainfall region (600 – 800 mm).

4.2.2 Sampling

Phloem sap typically flowed freely from small incisions (approximately 3 cm long) made in the bark of the main stem of trees to cambium depth (3 - 5 mm deep, depending on bark thickness), using a clean, sharp razor blade, with incisions made not less than 10 cm apart. Deeper incisions resulted in sap being sucked back into the transpiration stream of the xylem, while incisions that were too shallow failed to bleed, simply because sieve tubes had not been severed. Sap flow usually stopped about 10 s after incision, with no significant decrease in total sugar concentration. The spontaneous bleeding of sugar-rich sap was assumed to originate from cut sieve elements under osmotic pressure (Milburn, 1975).

As a general procedure, one bulk sample of sap was taken for every plot by collecting a 20 - 30 µl sample from each of ten trees with microcapillary tubes and bulking the sap in a 1.5 ml Eppendorf vial. Samples were quickly frozen in the field and later stored at -20 °C to await analysis. For the spring and autumn bulk samples, 30 - 40 µl of sap per tree were collected to allow for complete solute analysis. Sap samples were also collected from individual trees from a number of plots to examine within-plot variability in phloem sap composition. Within-tree variation in sugar concentrations was assessed by sampling six trees at 30 cm height intervals from one plot (K14) in January, 1996, while within-stem variation in concentrations of amino acids was assessed by sampling five uniform trees from the 1995 Klopper plantation at stem heights of 0 - 1m, 1 - 2 m, and 2 - 3 m above ground in November 1996. All other phloem samples were collected at a stem height of 1.0 - 2.0 m above ground, i.e. approximately in the mid-stem region.

Sap 'yield' was estimated by using the simple technique of collecting all sap which exuded from an incision for a 10 s period in microcapillary tubes and measuring the volume obtained. This was repeated for 10 cuts per tree, spaced at 10 cm height intervals (1.0 - 2.0 m stem height), for 10 trees per plot, giving a total sap yield per plot from 100 incisions.

Diurnal variation in sugar concentration and sap yield was also assessed. A thermometer, hung close to the main stem and away from direct sunlight, was used to measure air temperature at the time of collection of each sample.

Apart from smaller trials to assess variability in sap yield and sugar concentration, the main sampling periods for this study were from 10th November to 21st December 1995 (referred to as 'spring'), and from 19th March to 10th April 1996 (referred to as 'autumn'). Plots within each plantation were sampled within a three-day period of fine weather.

4.2.3 Phloem sap analysis

Concentrations of total sugars were determined in the field on a sub-sample of phloem sap immediately upon collection using an Erma temperature-compensated hand refractometer, calibrated in the range 0 - 32 % sucrose equivalents (w/v). The refractometer was zeroed several times a day with distilled water. A number of bulk samples were re-measured in the laboratory after thawing to room temperature (20 °C), but no significant differences were found between field and laboratory determinations. This technique allowed a large number of samples to be analysed simply and at low cost.

Bulk sap samples were analysed for sugars, organic acids and amino acids using high performance liquid chromatography (HPLC). Sugars and organic acids in phloem sap were analysed by isocratic separation in a Waters modular HPLC system with 0.008 N sulphuric acid eluent flowing at 0.50 ml min^{-1}. The columns used were a Biorad organic acid HPX-87H (300 mm x 7.8 mm) in series with a Biorad fast acid HPAH (100mm x 7.8 mm), with an in-line Biorad cation-H guard column (40 mm x 4.6 mm). Run temperature was 27 °C. Sugars were detected on a Waters 410 Refractive Index (RI) Detector at 38 °C cell temperature. Identification and quantitation of sugars was by comparison with characteristic retention times using external standards and checked by UV_{210}/RI ratios. Glucose and fructose were not determined due to matrix smearing from the dominant sucrose and raffinose peaks. Organic acids were detected with a Waters 996 PDA over the range 205 - 220 nm, using 210 nm as a quantitation wavelength. Identification of organic acids was by comparison with external standards and by spectral likeness and purity matches to a library of standards. Only citric, malic, shikimic and fumaric acids could be positively identified.

Amino acids were analysed using a Waters physiological amino acid modular HPLC, with separation of amino acids by a programmed pH gradient using three buffers (lithium citrate pH 2.65, lithium borate pH 9.70, and lithium hydroxide (0.2N) pH 13.2) running at 0.35 ml min^{-1}. The column used was a physiological lithium column (AA-503, 125 mm x 4.6 mm) with an in-line guard column (GC-503, 24 mm x 4 mm). Run temperature was 34 °C. Amino acids were reacted with ninhydrin colour reagent after elution from the column at 0.20 ml min^{-1}, in a nitric acid passivated SC coil (10 mm x 0.25 mm) at 130 °C for approximately 45 s. Peaks were quantified using a Waters 490E 4 channel, multi-wavelength UV-VIS detector at 546, 436, 405 and 500 nm. Most amino acids were quantified on the basis of absorption at 546 nm; proline was quantified using absorbance at 436 nm. Amino acids were identified from characteristic retention times, and where necessary, from multiple wavelength ratios. Concentration of amino acids was quantified against internal standards (NMA, SMC and NLEU) and external standards as required.

The mineral composition (P, K, Ca, Mg, S, Na, Cu, Zn, Mn and Fe) of phloem sap was determined using either Inductively Coupled Plasma Spectrometry (ICP) (McQuaker *et al.*, 1979) or, in the case of some potassium analyses, Atomic Absorption Spectrometry (AAS). There was no appreciable difference between results obtained by either method. In general, 200 µl of sap was accurately diluted to 10 ml with MQ water, but in some cases where inadequate sample was available, 150 or 100 µl sap was used. All blanks and standard solutions for major elements were made up in an equivalent sucrose solution (5.0 mg ml^{-1}). The diluted samples were then analysed by ICP. Slightly different dilutions and concentrations of sucrose were used for AAS.

4.2.4 Measurement of tree growth and parrot damage

The diameter at breast height over bark (DBH) was measured (using a diameter-calibrated measuring tape) on all trees in each plot when the plots were established in November - December 1995, and again in March - April 1996. A number of plots were also measured in January 1996.

Parrot damage was assessed for all plots in March/April 1996 by estimating the total length of bark stripped (TSL) on the main stem above a stem height of 2.5 m, and by the number of trees damaged per plot (TD) (see Chapter 2). This ensured that only those stems that had been damaged between November and March would be recorded.

4.3 Results

4.3.1 Typical composition of phloem sap

Total sugar concentrations in phloem sap collected from the mid-stem sampling position were typically in the range from 21 - 26 % (w/v), but concentrations as little as 15 % (w/v), and as great as 32 % (w/v), were recorded. In all samples of phloem sap analysed by HPLC, sucrose was the dominant sugar, but a significant proportion (maximum 33 % on mM basis) was also present as raffinose. Glucose and fructose were present in small concentrations but were difficult to quantify and are not reported independently. Detailed analyses suggested that the combined concentration of glucose + fructose was less than 5 % (w/v) of the concentration of sucrose + raffinose (Prof. J. Pate, pers. comm.). The total concentration of sugars determined by HPLC (w/v) was almost identical to that measured using the refractometer. The ratio of total sugar concentration measured using the refractometer, to that measured using HPLC, from 28 plots was 0.99 ± 0.005.

Total amino acid concentrations varied considerably among samples, and ranged from 0.7 - 24.2 μmol ml^{-1} for all samples collected in spring and autumn. Almost a 30-fold variation in the ratio of amino acids to sugars (μmol:mmol) was found in autumn (Table 4.1). Glutamine was the dominant amino compound in phloem exudate, accounting for up to 71 % of all amino compounds analysed. The concentration of glutamine was strongly related to the concentration of total amino acids (Fig. 4.2). The next most abundant amino compounds in order of importance were glutamic acid, phenylalanine, aspartic acid, glycine, alanine, serine and lysine. The concentrations of all amino compounds increased as glutamine concentration increased, with the notable exception of proline.

The proportion of glutamine was also positively related with both arginine and lysine, but negatively correlated with twelve other amino compounds, including aspartic acid, threonine, serine, glutamic acid, proline, glycine and alanine.

Only four organic acids were positively detected in the phloem sap of *E. globulus*, which were, in order of abundance, malic, citric, shikimic and fumaric acids. Several other peaks were found which could not be matched in terms of spectral likeness with a library of standards or with chromatographic behaviour of external standards. Total organic acid concentrations for all seasons ranged from 1.5 - 10.0 μmol ml^{-1}.

Total concentrations of carbon and nitrogen (μg ml^{-1}) in phloem sap were determined from the concentrations of individual sugars, amino acids and organic acids. A wide range of C:N values was found in autumn across all sites, with values ranging from 200 - 8199 (Table 4.1).

Table 4.1
Concentrations of total amino acids, total sugars, total organic acids and inorganic elements, and concentration ratios of solutes in phloem sap, sampled from 22 plots at the Klopper, East and Naranbee plantations in autumn, 1996.

Component	Mean	Range
Amino acids (µmol ml^{-1})	11.0	0.72 - 20.2
Sugars (µmol ml^{-1})	716	614 - 812
Organic acids (µmol ml^{-1})	5.1	2.6 - 10.0
K (µg ml^{-1})	1320	340 - 1985
Na (µg ml^{-1})	320	85 - 945
Mg (µg ml^{-1})	63	35 - 89
Ca (µg ml^{-1})	11	5 - 20
Fe (µg ml^{-1})	5.4	0 - 19.3
Mn (µg ml^{-1})	3.4	0 - 13.8
Zn (µg ml^{-1})	1.3	0.3 - 9.2
S (µg ml^{-1})	193	40 - 1985
P (µg ml^{-1})	59	35 - 130
Ratios		
Amino acids:Sugars ((µmol mmol^{-1})	17.1	1.1 - 30.6
C:N ratio (µg µg^{-1})	1116	200 - 8199
K:Na (µg µg^{-1})	7.3	0.4 - 20.7

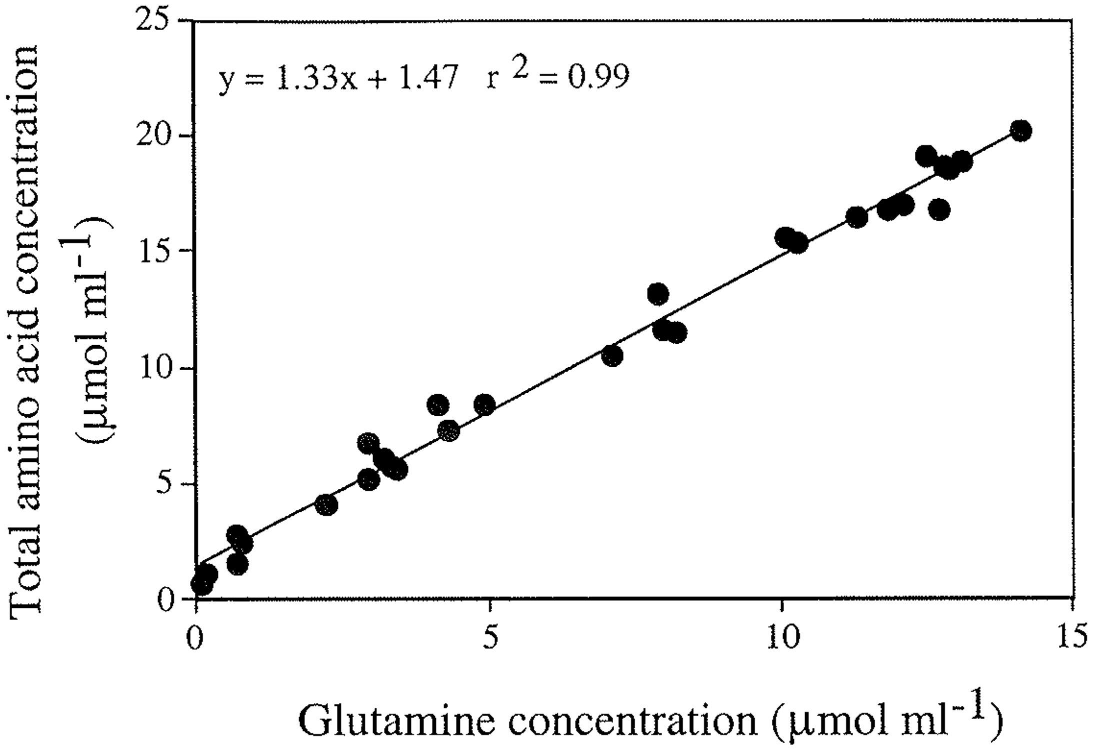


Figure 4.2 Relationship between the concentrations of glutamine and total amino acids in phloem sap of *E. globulus* sampled from 29 plots in autumn.

Potassium was the dominant cation in the phloem in all but two plots, followed by sodium, magnesium and calcium (Table 4.1). Magnesium did not vary greatly, and calcium concentrations were generally low. Iron, manganese, zinc and copper were present in low, but variable concentrations, and were undetectable in several samples. Sulphur showed a 10-fold variation in concentration, whereas there was little variation in phosphorus concentrations. Potassium concentrations were inversely and significantly ($P<0.001$) related to sodium concentrations (Fig.4.3), with a 50-fold range in K:Na ratios. In two plots (K15 and E3), the concentration of sodium in phloem exceeded the concentration of potassium.

4.3.2 Variation in sap yield and content with stem height

Maximum sap yield was obtained from the mid-stem sampling position, i.e. 1 - 2 m above ground for one- to two-year-old *E. globulus*. Sap yield from incisions made near the stem apex was generally very small and this limited the number of analyses that could be completed.

Total sugar concentrations varied considerably with stem height, and were least towards the stem apex (Table 4.2). Concentrations were greatest at approximately one-third of stem height, where the corresponding canopy leaf area was greatest, and decreased in both upwards and downwards directions, suggesting bi-directional sap flow from a central loading region towards ‘sinks’ in both root and shoot apices. The maximum difference in concentration between any two points on a stem was about 5 % (w/v, Table 4.2).

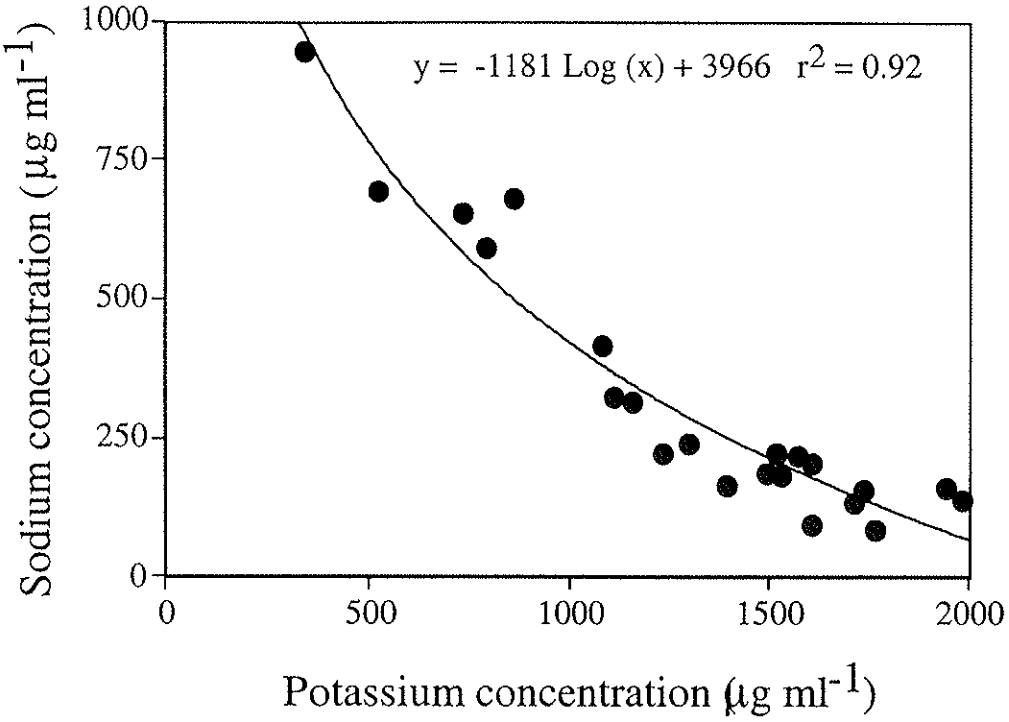


Figure 4.3 Logarithmic relationship between potassium and sodium concentrations in phloem sap sampled from 22 plots in the Klopper, East and Naranbee plantations in autumn.

Table 4.2
Sugar concentrations (% w/v) of phloem sap sampled at 30 cm intervals up the main stem of six similar sized trees in one plot (K14), at the Klopper plantation on 12th January, 1996. Concentrations were determined using a temperature compensated refractometer as total sucrose equivalents (w/v). Maximum sugar concentrations within a stem are shown in **bold** type. Time and temperature of phloem collection are shown. Mean tree height and diameter at breast height for the six trees were 4.60 ± 0.14 m and 5.1 ± 0.22 cm, respectively.

Height of sampling (m)	Tree number					
	1	2	3	4	5	6
	Time (hours)					
	0630	0730	0930	1000	1515	1610
	Temperature (°C)					
	16	17	24	25	26	27
3.0	-	17.6	17.2	21.2	21.2	22.8
2.7	18.0	21.4	19.0	20.8	22.2	22.8
2.4	18.4	19.6	17.4	20.8	23.4	22.0
2.1	18.4	19.6	18.8	21.4	23.4	22.6
1.8	18.0	20.2	18.8	22.0	**24.6**	24.0
1.5	19.4	**22.4**	**20.0**	22.0	24.0	**24.2**
1.2	**21.6**	22.0	19.2	22.0	23.0	23.8
0.9	**21.6**	21.0	19.6	**22.8**	**24.6**	**24.2**
0.6	21.0	21.6	19.0	22.4	**24.6**	23.8
Mean	19.6	20.6	18.8	21.7	23.6	23.4

In contrast to the situation for sugar gradients, total amino acids increased almost two-fold with stem height (see also Pate *et al.*, 1998). Bulk samples collected from five uniform trees in the 1995 Klopper plantation in November 1996 had considerably greater concentrations of amino acids (25.9 μmol ml^{-1}) in the upper stem sampling position than in the basal position (18.0 μmol ml^{-1}). Most of the difference in total amino acid concentration with stem height was accounted for by glutamine. While amino acid concentrations generally increased with stem height, some trees showed the reverse. For example, total amino acid concentrations decreased from 8.2 μmol ml^{-1} at 0 - 1 m, to 5.5 μmol ml^{-1} at 2 - 3 m in a single tree with particularly high sap flow near plot K2. The total concentration of amino acids in this tree was somewhat less than in the earlier example, and suggests that the magnitude and direction of amino acid gradients in individual trees may depend on relative demands of roots and shoots for nitrogen at the time of sampling.

4.3.3 Temporal variation in sap yield and content

Diurnal variation

Sugar concentrations and sap yields were measured for three sites at the Klopper plantation, at four times on one day in January 1996. For this study, a unique set of 10 trees, of similar mean DBH, was sampled near the defined plot at each time period to avoid the possibility of sap flow being affected by incisions made earlier in the day. Sugar concentrations were not significantly affected by sampling time (Table 4.3), even though temperatures increased by more than 10° C during the day. Concentrations of sugars varied significantly among sites ($P<0.01$), and were greatest at K14 (Table 4.3), but there were no significant differences in sugar concentrations among trees within any of the sites.

Table 4.3
Diurnal variation in phloem sugar concentration (% w/v) and sap yield for three plots at the Klopper plantation, sampled at four times on 9th January 1996. Sugar concentrations are mean plot values (n = 10 trees) measured by refractometer. Sap yield is the total volume of sap collected from ten incisions per tree with ten trees per plot.

SITE	Time h	Temp °C	% Sucrose	Sap yield µl
K1	0530 - 0700	17	21.9 ± 0.2	138 ± 31
	0830 - 1000	22	22.2 ± 0.2	82 ± 17
	1130 - 1300	29	21.5 ± 0.5	88 ± 24
	1430 - 1600	28	22.1 ± 0.4	39 ± 12
		Mean	21.9 ± 0.2	87 ± 12
K12	0530 - 0700	16	22.2 ± 0.1	61 ± 24
	0830 - 1000	20	21.9 ± 0.3	40 ± 9
	1130 - 1300	28	21.4 ± 0.7	20 ± 5
	1430 - 1600	28	22.8 ± 0.6	42 ± 15
		Mean	22.1 ± 0.3	41 ± 8
K14	0530 - 0700	16	23.4 ± 0.1	111 ± 3
	0830 - 1000	23	23.0 ± 0.4	61 ± 23
	1130 - 1300	29	23.6 ± 0.3	73 ± 19
	1430 - 1600	28	23.5 ± 0.6	45 ± 5
		Mean	23.4 ± 0.2	72 ± 11

Sap yields were greatest early in the morning, and decreased during the day with little change between 0830 and 1300 h (Table 4.3). The decline in yield with time was significant at only one site, K1 ($P<0.05$). Mean sap yield varied significantly among sites ($P<0.05$), and among individual trees within each site (0 - 32 μl sap tree^{-1}), but there was no significant relationship between sap yield and sugar concentration for all trees (n = 87).

Seasonal variation

Sugar concentrations increased in plots at the Klopper and East plantations between spring and autumn, but not at the Naranbee plantation (Table 4.4). However, the overall increase in mean sugar concentration between seasons was less than 2.0 % (w/v).

Within the Klopper plantation, sugar concentrations (mM) were significantly greater in autumn than in spring ($P<0.01$). These differences were mainly due to increased concentrations of sucrose (from 482 mM to 541 mM), with no significant change in raffinose concentration (162 mM and 154 mM). As a result, the molar ratio of raffinose to sucrose tended to be less in autumn (0.29), than in spring (0.34), but this difference was not significant ($P = 0.063$). Trees at the high rainfall East plantation generally had greater raffinose:sucrose ratios (0.35 - 0.54) in autumn than at the lower rainfall plantations, which suggests that both total sugar concentration and the raffinose:sucrose ratios may vary with soil water availability.

Amino acid concentrations varied considerably among plots within the Klopper plantation in both seasons (Fig. 4.4), but did not differ significantly between seasons. Concentrations of amino acids were markedly less in some plots (e.g. K7, K8, K15 and K16) in autumn than in spring, whereas in other plots of well grown trees (e.g. K1, K2 and K4), concentrations were greater in autumn.

Table 4.4
Mean phloem sugar concentrations assessed by refractometer (% w/v), in three plantations, each with ten trees per plot (n). Sugar concentrations were measured in 'Spring' (November – December) 1995 and 'Autumn' (March – April) 1996. The significance of differences between seasons is indicated (****P*<0.001, ***P*<0.01, **P*<0.05, ns not significant).

	Klopper (17)	East (6)	Naranbee (6)	All plots (29)
Spring	23.9	21.1	23.0	23.2
Autumn	26.3	22.7	23.8	25.0
P	***	*	ns	***

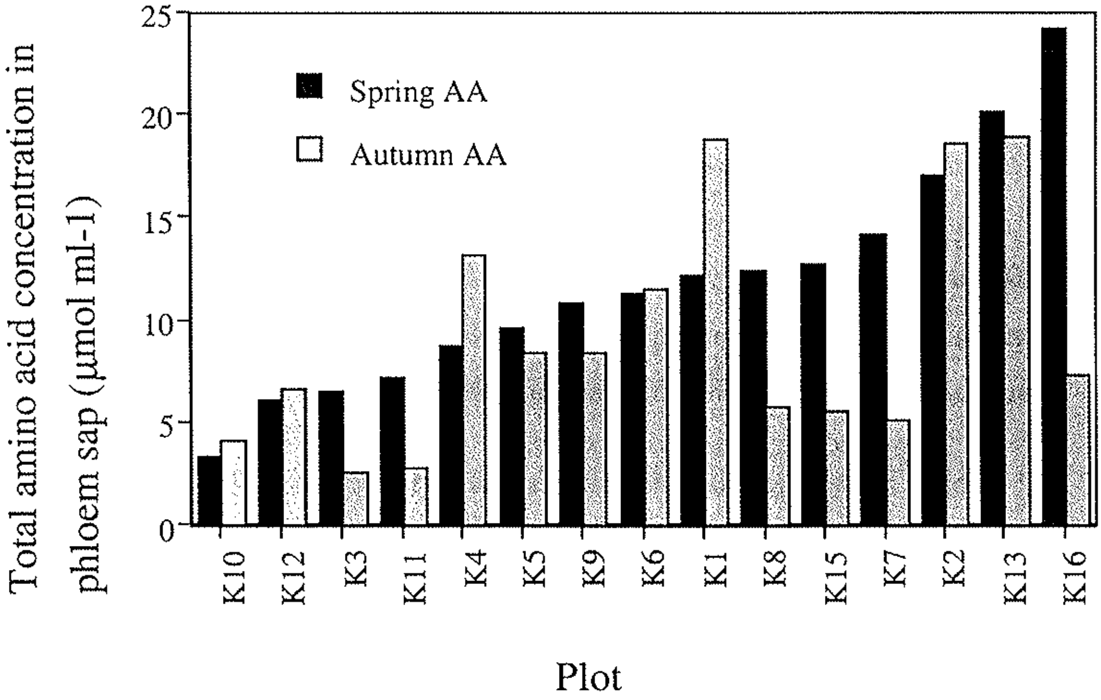


Figure 4.4 Concentration of total amino acids in phloem sap of *E. globulus* sampled from 15 plots in spring 1995, and in autumn 1996 at the Klopper plantation.

Large variations in amino acid composition were measured between plots at the Klopper plantation in both seasons (Table 4.5a). Glutamine concentrations varied almost ten-fold while other amino acids were less variable. For most amino acids, mean concentrations were less in autumn than spring and this difference was significant for glycine, alanine, valine, methionine, isoleucine, leucine, histidine and lysine. Cystine, tryptophan, DH-lysine and ornithine were present in spring but were not detected in autumn.

Two plots of particular interest, K3 and K11, with low concentrations of total amino acids, had relatively high concentrations of proline (0.08 and 0.12 μmol ml^{-1}, respectively). These trees yielded little sap and were obviously water stressed. In contrast, plots E3 and N5, with the least concentrations of total amino acid (1.12 and 0.72 μmol ml^{-1}), had low proline concentrations (i.e. 0.03 and 0.01 μmol ml^{-1}, respectively). Trees in these plots were growing on infertile sand and showed symptoms of severe nutrient deficiency rather than water stress.

Total concentrations of organic acids were greater in autumn than in spring ($P<0.05$) at the Klopper plantation (Table 4.5b), due mainly to greater concentrations of malic and citric acids. Variation in organic acid concentrations among plots was not as pronounced as with amino acids. Concentrations of malic acid in autumn were greatest in plots K3 and K11 (9.96 and 8.27 μmol ml^{-1}, respectively), which also had relatively large concentrations of proline (see above).

Mean C:N ratios in sap from plots at the Klopper plantation were greater in autumn (692) than in spring (384) ($P = 0.06$). There was also a greater range in C:N values in autumn (226 - 2265) than in spring (168 - 697) at this plantation.

Mean potassium concentrations in phloem sap at the Klopper plantation were 1411 μg ml^{-1} in spring compared with 1676 μg ml^{-1} in autumn, but the difference was not significant. Potassium concentrations were least at K15 in both spring (398 μg ml^{-1}) and in autumn (654 μg ml^{-1}, Fig. 4.5).

Table 4.5
Seasonal variations in concentrations of [a] amino acids (nmol ml^{-1}) and [b] organic acids (μmol ml^{-1}) in phloem sap from 15 plots at the Klopper plantation. Significant differences between seasons for each compound are indicated (****P*<0.001, ***P*<0.01, **P*<0.05).

	Spring		Autumn	
Compound	Mean	Range	Mean	Range
[a]				
Aspartic acid	498	73 - 1190	358	122 - 725
Threonine	102	46 - 253	86	33 - 146
Serine	319	43 - 535	272	95 - 445
Asparagine	109	14 - 266	63	13 - 216
Glutamic acid	988	263 - 1689	941	406 - 1562
Glutamine	7009	1678 - 16439	5631	749 - 13114
Proline	39	18 - 80	47	0 - 117
Glycine	471	213 - 848	291**	103 - 448
Alanine	371	94 - 870	241*	110 - 486
Citrullin	48	0 - 209	21	0 - 50
Valine	61	28 - 123	18***	7 - 39
Cystine	5	0 - 9	0	-
Methionine	43	18 - 91	25*	8 - 68
Isoleucine	26	11 - 58	11***	4 - 26
Leucine	11	5 - 33	4*	0 - 11
Tyrosine	136	36 - 274	97	21 - 219
Phenylalanine	530	165 - 905	487	231 - 983
g-a Butyric acid	79	13 - 469	76	19 - 317
Histidine	121	25 - 300	55**	9 - 131
Tryptophan	144	39 - 362	0	-
DH-Lysine	100	22 - 159	0	-
Ornithine	43	10 - 110	0	-
Lysine	296	55 - 763	144*	12 - 405
Arginine	191	0 - 526	165	0 - 583
[b]				
Citric	0.99	0.46 - 1.26	1.26*	0.78 - 1.84
Malic	2.73	0.93 - 4.37	3.80*	2.11 - 8.80
Shikimic	0.14	0.08 - 0.20	0.12	0.06 - 0.21
Fumaric	0.12	0.02 - 0.26	0.16	0.07 - 0.33

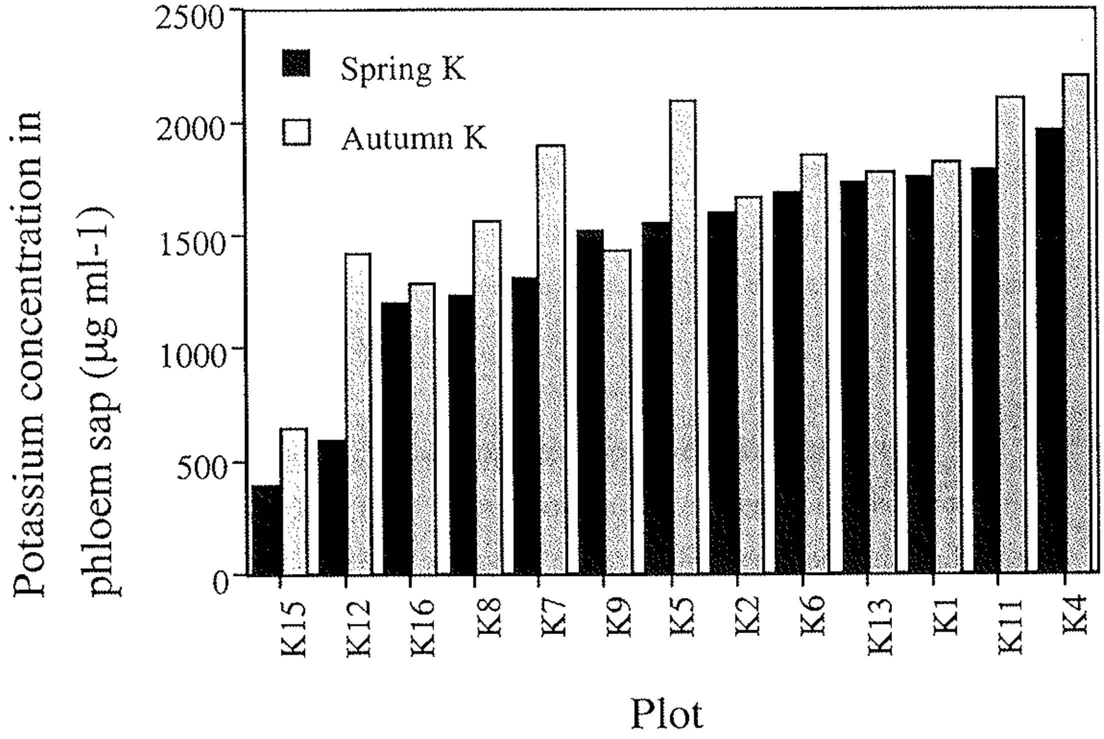


Figure 4.5 Potassium concentrations in phloem sap of *E. globulus* sampled in spring 1995 and in autumn 1996 from 13 plots at the Klopper plantation.

4.3.4 Variation in sap yield and content with tree growth

Tree diameter (DBH), phloem sap yield and sugar concentration were assessed for 20 plots (200 trees) from five plantations during a three week period in January 1996. This study included two additional plots from a plantation near Augusta (McDonald), and two plots from a plantation near Margaret River (Connolly) in the high rainfall region of Western Australia. At the East plantation in the high rainfall region, there was no relationship between DBH and sugar concentration ($n = 60$), despite large differences in tree growth (2.0 - 7.8 cm DBH). However, for all plots measured, sugar concentrations tended to decrease with increasing DBH ($P = 0.06$).

In contrast, mean sap yields for all plots were positively and significantly ($P<0.001$) related to DBH (Fig.4.6). As found in the earlier trial at the Klopper plantation (see Table 4.3), there was no relationship between sap yield and sugar concentration. In this trial, neither sampling time nor temperature had any significant effect on sap yield or sugar concentration.

In autumn 1996, sugar concentrations in phloem sap sampled from plots in the main trial were inversely related to DBH ($P<0.001$) when five plots of small, obviously nutrient deficient trees (E1, E3, E6, N5 and N6) were discarded from the regression analysis (Fig. 4.7a). Conversely, there was a strong positive relationship ($P<0.001$) between amino acid concentration in phloem and tree growth (Fig. 4.7b) for all plots ($n = 29$) in autumn. Least concentrations of amino acids (<2.0 μmol ml^{-1}) were measured in three plots of small trees (E3, N5 and N6) that had nutrient deficiency symptoms (see Chapter 5). Concentrations of amino acids were also low in other plots in which tree growth appeared to be adversely affected by poor soil water availability, rather than poor nutrient supply (e.g. K3 and K11) (see Fig. 4.4). Not surprisingly, C:N ratios of phloem sap were also shown to be strongly related to DBH (Fig. 4.8), with extremely high C:N values being recorded in trees at N5 (8200) and E3 (4600). Healthy trees that had no symptoms of either nutrient deficiency or water stress generally had C:N values less than 500.

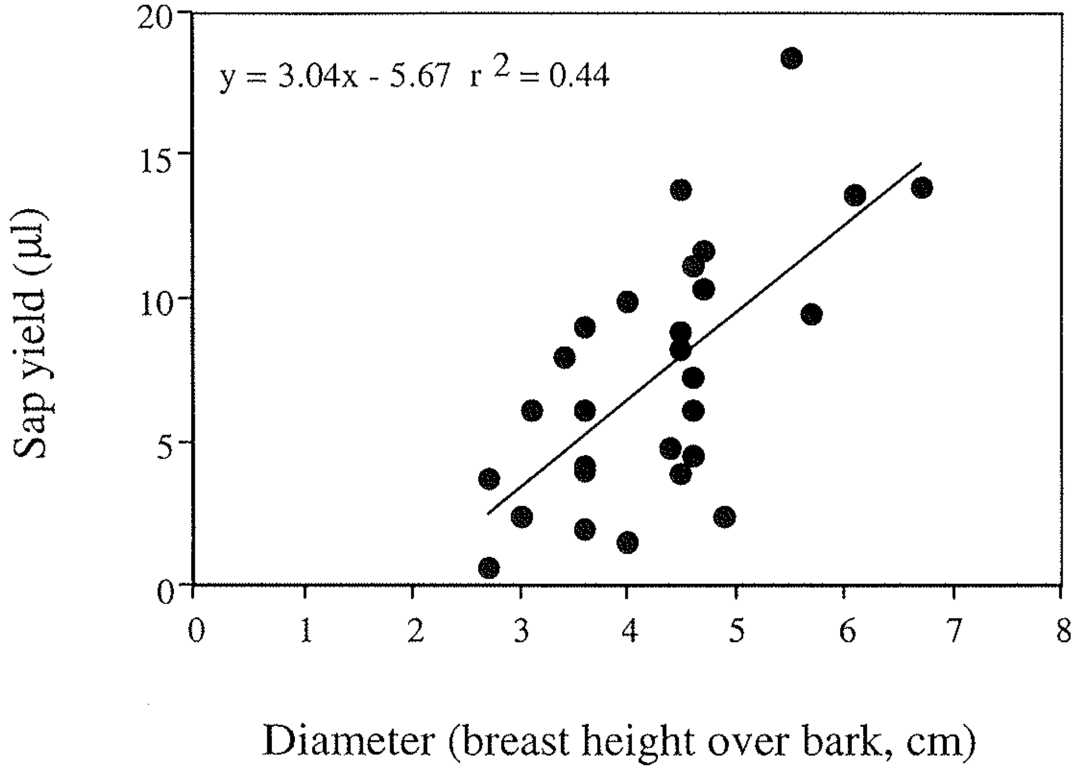


Figure 4.6 Relationship between diameter at breast height (DBH) and mean sap yield from 19 plots of *E. globulus* sampled from five plantations across the south-west of Western Australia in January 1996.

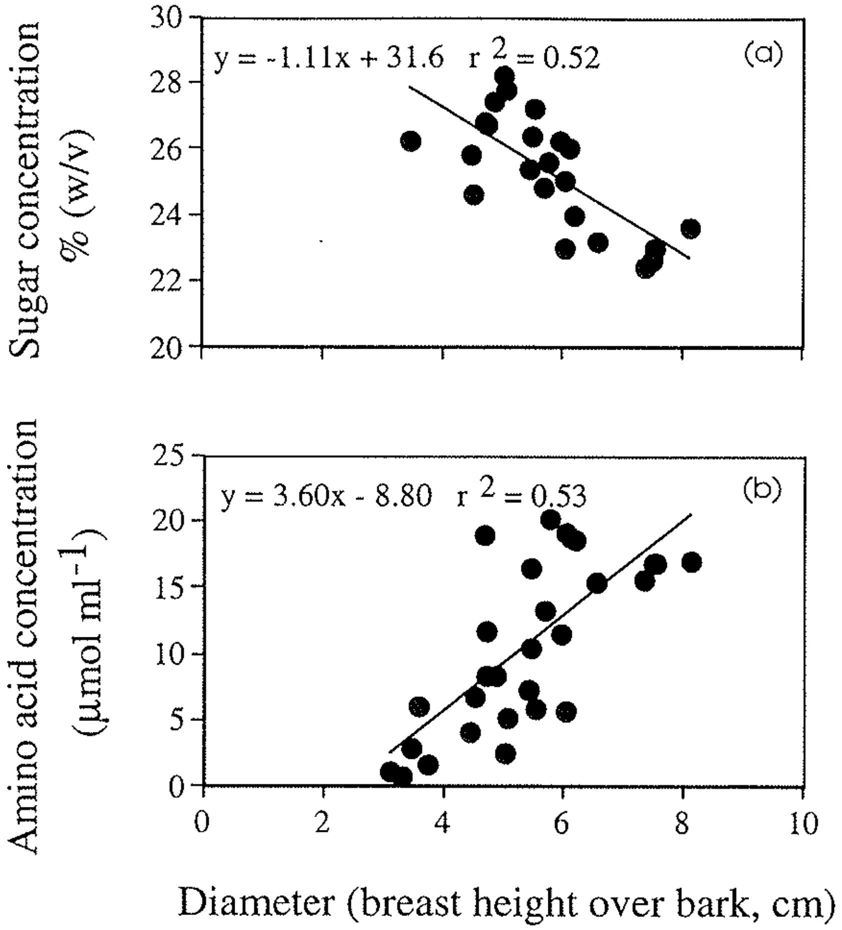


Figure 4.7 Relationships between diameter at breast height (DBH) and concentrations of (a) total sugars and (b) total amino acids in phloem sap sampled in autumn from 20-month-old *E. globulus* from the Klopper, East and Naranbee plantations.

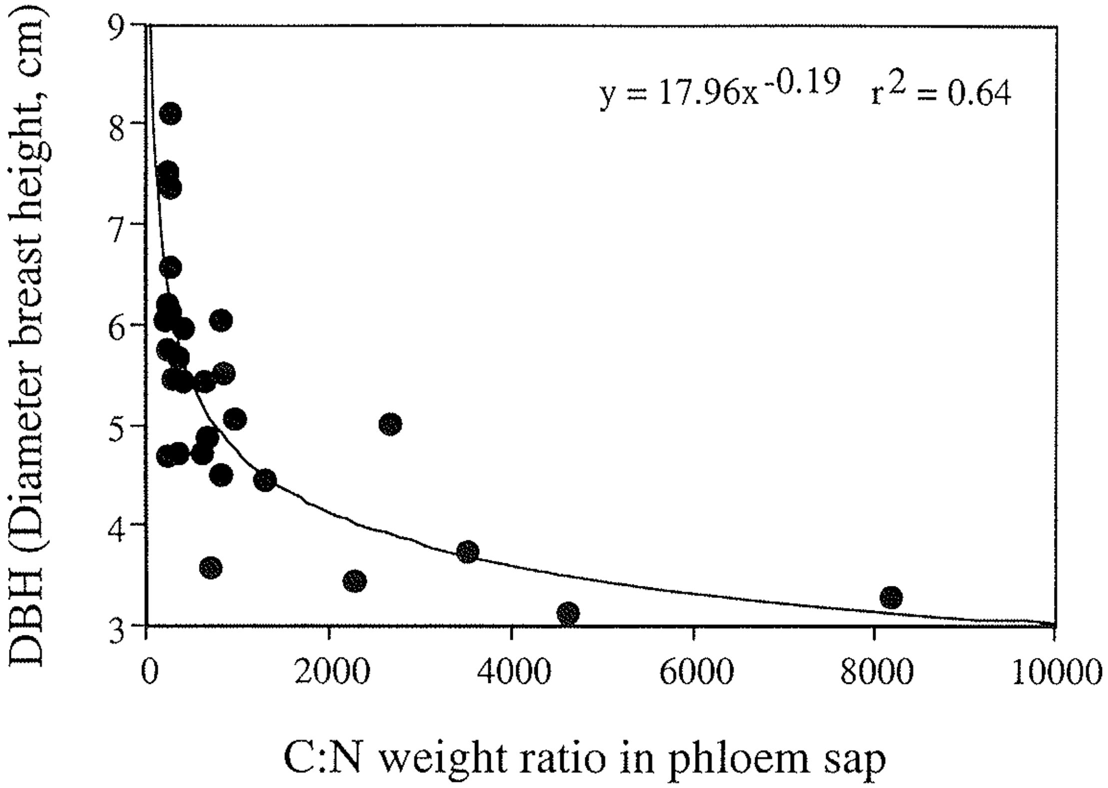


Figure 4.8 The relationship between diameter at breast height (DBH) and the C:N ratio in phloem sap sampled from 29 plots of *E. globulus* in the Klopper, East and Naranbee plantations in autumn 1996.

In autumn, potassium concentrations in phloem sap from all plots ranged from 340 - 1985 μg ml^{-1}. Trees in one plot at the East plantation (E3, DBH 3.1 cm) with the least potassium concentration (340 μg ml^{-1}) had visual symptoms of potassium deficiency. Trees in N5 and N6 (DBH 3.3 and 3.8 cm, respectively) also had low concentrations of potassium in the phloem. A significant relationship ($P<0.001$) between potassium concentration in phloem sap and mean DBH was evident in autumn (Fig. 4.9), and suggested, along with visual symptoms and foliar analyses (data not presented), that potassium may have been limiting growth in many trees. As would be expected from Fig. 4.7b and Fig. 4.9, potassium concentrations were also strongly related to the concentration of total amino acids in the phloem ($r^2 = 0.660$, $P<0.001$). Although potassium concentrations varied widely among sites, there was no relationship between the concentrations of potassium and total sugars in phloem sap.

4.3.5 Assessment of parrot damage

Twenty-eight parrots had damaged trees in 15 of the 29 plots in the period from November 1995 to the time of assessment in March/April, 1996. Damage was greatest in plots K17, N1 and N2, which had a mean 'total strip length' (see Chapter 2) of more than 50 cm per tree. Phloem sap collected from all trees in these plots in autumn had relatively high concentrations of amino acids (>15 μmol ml^{-1}) and low C:N ratios (<250), whereas no damage was recorded in any plots with amino acid concentrations less than 5 μmol ml^{-1}.

Even though the abundance of parrots varied among plots, the proportion of trees damaged per plot (TD) was positively related to DBH ($r^2 = 0.22$, $P<0.01$), and to the concentration of amino acids in phloem ($r^2 = 0.26$, $P<0.01$). On the other hand, tree damage was weakly and negatively related to phloem sugar concentrations ($r^2 = 0.13$, $P = 0.06$). Eight out of nine plots with concentrations greater than 26 % (w/v) remained undamaged.

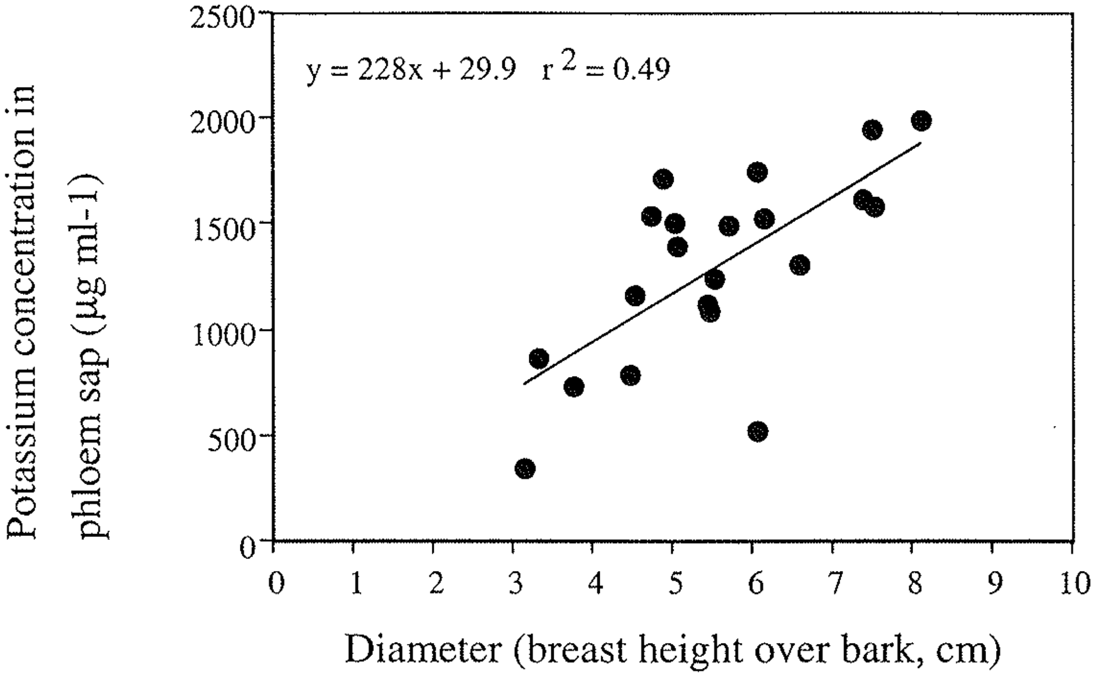


Figure 4.9 The relationship between potassium concentrations in phloem sap and diameter at breast height (DBH) in 22 plots at the Klopper, East and Naranbee plantations in autumn 1996.

4.4 Discussion

Yield and composition of phloem sap from *E. globulus* varied considerably, and was strongly related to growth of trees at the time of sampling. Greater differences were generally found within plantations than among plantations, or between seasons. The scale of spatial variability in sap yield and composition was consistent with previously determined patterns of parrot damage (see Chapter 2), whereas seasonality of damage is more likely to be determined by other factors, such as availability of alternative food resources and parrot breeding cycles.

Compared with other species growing near Perth, Western Australia, total sugar concentrations in *E. globulus* were generally greater than those found in vigorously growing three-year-old *Nicotinia glauca* (15.5 - 16.8 % w/v) (Hocking, 1980) or in six-year-old *Banksia prionotes* (18.4 % w/v) (Jeschke and Pate, 1995), but were similar to those in two-year-old *Robinia pseudoacacia* (23.0 % w/v) (Atkins *et al.*, 1991). A greater range of total sugar concentrations (18 - 37 % w/v) was recorded for *E. viminalis* in studies related to sap feeding by gliders (Goldingay, 1987), but the method of sap collection may have allowed the sugar concentrations to increase through evaporation.

Sugar concentrations in phloem sap in this study were similar to those found in autumn in nectar (17 - 28 % w/v) in nearby native trees (*Corymbia calophylla* and *Eucalypus patens*), on which parrots feed avidly. This similarity in sugar concentrations suggests that parrots might readily substitute phloem sap for nectar when nectar is in short supply. Although most trees are damaged in autumn at a time when sugar concentrations in phloem sap are greatest, parrots may be more strongly influenced by the volume of nectar and phloem sap than by its sugar concentration.

For example, on one occasion in February 1997, I observed parrots feeding excitedly in several heavily flowering *E. patens* growing in a moist gully, which had copious quantities of nectar (up to 100 µl nectar per flower) yet the concentration of sugar in this nectar was only 11 - 15 % (w/v). This is despite a clear preference shown by parrots in aviary trials for sweeter solutions (pers. observ.). In these aviary trials (see Chapter 3 section 3.4), parrots consistently consumed greater volumes of 25 % (w/v) sucrose solution (mean 9.1 ml bird^{-1} day^{-1}) than 15 % (w/v) sucrose solution (mean 4.2 ml bird^{-1} day^{-1}) offered in two choice tests. However, the volume of solution offered in the aviary was not limited, whereas the volume of sap exuding from wounds on eucalypt stems can vary greatly.

Conceivably, the total mass of sugar extracted per feeding event, or per centimeter of bark stripped, is of greater value to parrots than its sweetness. For example, a sap yield of 60 μl at 25 % (w/v) sucrose equivalents would provide 15 mg sucrose, whereas 30 μl at 30 % (w/v) sucrose equivalents would provide only 9 mg sucrose. Given the greater level of variability between trees in sap yield than in phloem sugar concentration, it is likely that faster growing trees, with higher sap yields and moderate sugar concentrations would provide more sugar per feeding event than slower growing trees with low sap yields and high sugar concentrations.

Nectar and phloem sap may also provide an alternative source of drinking water, reducing the need for parrots to find fresh water when surface water is scarce or is too saline, or when access to the surface water might predispose the birds to an unacceptably high risk of predation. Parrots substituted up to 50 % of their total daily water intake (between 10 and 30 ml bird^{-1}) with 25 % sucrose solution (up to 12 ml bird^{-1}) when water was offered as one of the solutions in the 'two choice' aviary trials mentioned above. In other trials where water was not offered, parrots consumed over 30 ml of 25 % sucrose solution per day and appeared not to be unduly stressed. This suggests that parrots may be also be seeking the phloem sap in vigorously growing trees as a source of water during the hot and dry summer, and may explain their preference for trees with greater sap flow.

Yellow-bellied gliders are also known to select trees for sap-feeding that produce a significantly greater yield of sap (Henry and Craig, 1984; Goldingay, 1987), have 'vigorous' growth (Craig, 1985; Sullivan *et al.*, 1986; Mackowski, 1988), or which occupy fertile gullies and creekflats (Kavanagh, 1987). By contrast, Goldingay (1987) found no relationship between sugar concentrations in the phloem sap of *E. viminalis* and the selection of trees for sap feeding by yellow-bellied gliders, despite large differences in sugar concentrations between individual trees.

Phloem exudation in *R. communis* was previously shown to be directly dependent on the level of water stress, whereby the rate of exudation decreased sharply when water was withheld (Hall and Milburn, 1973). This decrease in exudation rate was accompanied by a rise in sap concentration. Hocking (1980) also noted a correlation between sap yield and plant vigour. This effect may be associated with increased phloem width or live-bark thickness in vigorously growing trees (Carter and Blanchard, 1978; Kile *et al.*, 1982; Mackowski, 1988). Water stress in *E. globulus* is known to reduce the rate of diameter growth, and can lead to stem shrinkage (Hingston *et al.*, 1998; Downes *et al.*, 1999). Downes *et al.* (1999) attributed this stem contraction to the reversible movement of water primarily out of the cambial region and into the transpiration stream.

Water stress strongly affected cambial activity at the Eulup plantation near Mt Barker, where 'windows' of bark were cut out to observe the directionality of phloem sap flow (J. Pate, pers. comm.). During the period of greatest water stress, there was no sap flow and cambial tissues were quite dry and apparently inactive, but when the water stress was relieved by opening rains, the cambium became gelatinous and sap started to flow. In water-limited environments such as much of south-west Western Australia, it is not surprising that phloem exudation may be related to availability of soil water.

The ease with which the bark can be stripped from the stem will also depend on the degree of water stress. It is well known by horticulturalists that bark 'slips' more readily for grafting operations when cambial tissues are actively dividing and cell walls are thin, and newly formed tissues are easily torn (Hartmann and

Kester, 1975). Therefore, it seems that any adverse growing conditions, such as water stress, salinity, defoliation or low temperatures, which affect cambial activity, may cause the bark to tighten and be more difficult for parrots to remove.

Sucrose produced directly from photosynthesis, or from hydrolysis of storage starch, is important for cambial growth (Catesson *et al.*, 1995), and may vary seasonally in response to growth demands (Fisher, 1983). In the northern hemisphere, maximum concentrations of sucrose were recorded in midwinter in young stems of poplar (*Populus*) and dogwood (*Cornus sericea*) as a physiological response to low temperatures (Fege and Brown, 1984; Bonicel *et al.*, 1987; Ashworth *et al.*, 1993). However, for *E. globulus*, sugar concentrations were least in October and November, when shoot extension growth was maximal, and greatest in autumn as soil moisture limited growth, but there was no further increase in sugar concentration during the coldest months of July and August (Pate *et al.*, 1998). This suggests that, in our temperate climate, sugar accumulates when the demand for sugars for growth and cambial activity is low, rather than in response to low temperatures.

Raffinose was previously identified in phloem exudates in four species of *Eucalyptus* and in other species belonging to Myrtaceae (Zimmermann and Ziegler, 1975). Raffinose is known to have seasonal cycles in some woody species and appears to have a role in cold tolerance (Zimmermann and Ziegler, 1975; Ashworth *et al.*, 1993; Bachmann *et al.*, 1994). Since raffinose has very little sweetness compared with sucrose (pers observ.), it was thought that seasonal variations in the concentration of raffinose to sucrose might influence the attractiveness of the *E. globulus* stems to parrots. However, there was little variation in the ratio of raffinose to sucrose in this study over a wide range of sites, or over a whole year (Pate *et al.*, 1998).

Strong gradients in sugar concentrations with stem height were similar to those reported by Pate *et al.* (1998) for *E. globulus*, and to those recorded for other species (Milburn, 1974, 1975; Hocking, 1980). Both upward and downward gradients were found in *E. globulus*, with greatest concentrations in the mid-stem region where there was greatest leaf area. Sugar concentrations were least towards the stem apex that is most severely damaged by parrots, and by inference from the effects of growth on sugar concentrations (Fig. 4.7a), are likely to be least in the upper stems of the most rapidly growing trees within a plantation. Thus sugar concentration *per se* is unlikely to be the major determinant of tree selection by parrots.

This again suggests that either parrots are insensitive to the range of sugar concentrations recorded in *E. globulus* in this study, or that they are unable to access the phloem with greater sugar concentrations further down the stem. During the survey of almost 7000 trees for parrot damage (Chapter 2), I rarely saw fresh bark stripped on stems where the stem diameter was greater than 3 cm. This indicates that either the diameter of the stem in the mid-stem region was too wide for parrots to gain leverage, or the bark itself was too thick or did not strip easily.

In contrast, amino acid concentrations in autumn were positively related to tree growth and generally increased with stem height – clearly in keeping with patterns of parrot damage. Where conditions for growth were favourable, concentrations of amino acids in the phloem remained high during autumn. However, in some plots, and particularly at the Eulup plantation, growth and amino acid concentrations decreased markedly from spring to autumn as the level of water stress increased (Pate and Arthur, 1998). Even though amino acid concentrations tend to be greater in spring than in autumn, there is an abundance of other high protein and high-energy food in spring that dictates low rate of parrot damage (Ritson, 1995).

This is not to say that parrots can discriminate between trees on the basis of amino acid concentration alone, but it is possible that amino acid concentrations in phloem are likely to coincide with other features in stems, which together may be conducive to selection of stems by parrots. For example, trees with faster growth rates would be expected to have a thicker cambium with greater protein concentrations, as well as bark that strips more readily. Nitrogen concentrations in cambial tissues can be greater than 5 % N (or 31.2 % protein) (Mattson, 1980), providing an additional supply of protein to parrots at a time of year when the availability of other sources of protein may be limited.

The total concentrations of amino acids found here in phloem exudates in *E. globulus* (3 - 24 μmol ml^{-1}) during spring were considerably less than those recorded by Hocking (1980) for *Nicotiana glauca* (80 μmol ml^{-1}), and Atkins *et al.* (1991) for *Robinia pseudoacacia* (49 μmol ml^{-1}) for the same season, and by Hall and Baker (1972) for *Ricinus* (35 μmol ml^{-1}) and Ziegler (1975) for a variety of tree species (up to 138 μmol ml^{-1}), but was greater than that recorded for *Banskia prionotes* (5.8 μmol ml^{-1}) growing on deep white sand over an 18 month period (Jeschke and Pate, 1995). Total phloem amino acid to sugar ratio in *E. globulus* in this study during autumn ranged from 1.1 - 30.6 (μmol mmol^{-1}) compared with 10.8 for *B. prionotes*, 72.9 for *R. pseudoacacia*, 150 for *Ricinus* and 176.6 for *N. glauca* from the previously mentioned studies.

The concentrations of amino acids relative to sucrose were shown in previous studies with lupins to vary widely with availability of nitrate in the rooting medium (Atkins *et* al., 1979), and presumably, differences in supply of nitrogen in soils strongly influenced the concentration of amino acids found in phloem sap in the present study. These large differences in amino acid concentrations reflect much greater flexibility for loading amino acids into sieve tubes than for sugars, and this has significance in relation to the availability of amino acids for shoot or root growth.

The N- rich amide, glutamine, was the major amino acid in phloem samples of *E. globulus*, and accounted for most of the variability in total amino acid concentrations. Glutamine and the partner amide asparagine, are the predominant amino compounds in the phloem sap in other woody trees (Ziegler, 1975) as they are in the xylem sap of a variety of plants (Pate, 1980; Simpson, 1986) including eucalypts (Adams *et al.*, 1995). Glutamine is also the major amino compound (> 80%) in xylem sap of *E. globulus*, *E. regnans* and *E. nitens* (Adams and Atkinson, 1991; Adams *et al.*, 1995; Pate *et al.*, 1998), although Pate *et al.*

(1998) observed that arginine may exceed glutamine as a proportion of total xylem-N at certain times of the year.

Free arginine is known to accumulate in leaves in response to high nitrogen supply (Näsholm and Ericsson, 1990; Näsholm *et al.*, 1994), and in response to low supply of phosphorus supply (Rabe and Lovatt, 1986). Adams *et al.* (1995) suggested that arginine might accumulate in older senescing eucalypt leaves rather than being transported to younger leaves via phloem sap due to its relatively low mobility in phloem sap. However, in this study, appreciable amounts of arginine were present in phloem sap in this study in trees with high nitrogen status and high concentrations of glutamine in phloem sap. Arginine may be present in phloem sap as a storage molecule (Pate, 1973) and its presence may indicate when trees cannot use all the nitrogen acquired for growth Näsholm *et al.* (1997).

Interestingly, the concentration of amino acids (mainly glutamine) in xylem sap of *E. regnans* increased with increasing proportions of NO_3^-: NH_4^+ in the soil (Adams and Atkinson, 1991). The form of nitrogen applied as fertiliser also altered the asparagine : arginine ratio in apple stems (Tromp and Ovaa, 1979). In the present study, plantations of *E. globulus* were established on ex-farmland, which would be expected to show higher concentrations of nitrate-N than in typical forest soils. The extent to which the form of soil nitrogen affects the amino acid concentration and composition in phloem sap of *E. globulus* is certainly worthy of further study.

Parrots feeding on faster growing trees in autumn may also benefit specifically from proportionally greater concentrations of lysine. Lysine is an essential amino acid which is commonly deficient, or in an unavailable form in granivorous diets (Moir, 1994). This deficiency may be greater in autumn when there is a shortage of food sources that are rich in available lysine (i.e. unripe seeds and insects). It remains to be seen whether complementary effects of amino acid balance of diets are of significance in the nutrition of parrots.

The mineral composition of phloem sap in *E. globulus* varied greatly. In particular, potassium concentrations varied almost six-fold and were positively related to DBH, suggesting that a deficiency of potassium may have been limiting to growth of some trees. Increased availability of potassium within the plant enhances the loading of sucrose onto phloem and increases the translocation of photosynthates from leaves (Hartt, 1969; Doman and Geiger, 1979; Peel and Rogers, 1982; Cakmak *et al*, 1994), without significantly changing the phloem sucrose concentration (Mengel and Haeder, 1977). Thus high potassium concentrations may have contributed to the increased sap yield in faster growing trees in this study, in addition to the effects of water availability discussed earlier.

Potassium is the major cation in phloem of virtually all species so far studied (Hall and Baker, 1972; Ziegler, 1975; Hocking, 1980), and was the major cation in the phloem sap in all but two samples in *E. globulus*. In these two samples, the concentration of sodium exceeded that of potassium more than two-fold. Greater concentrations of sodium than potassium were also found in phloem from *Banksia prionotes* growing in very infertile white sand (Jeschke and Pate, 1995). In the present study, sodium and potassium were inversely related, and it appears that sodium may have a sparing role when the external concentration of potassium is low (e.g. plot K15). It may also indicate that *E. globulus* has a poor ability to exclude sodium, and explain its relatively low salinity tolerance

4.5 Conclusions

The composition of phloem sap in *E. globulus* varied considerably between sites and seasons, and among trees with differing growth rates. This variation owed much to apparent differences in soil water and nutrient availability. However, in all cases, concentrations of total sugars in phloem sap were sufficiently high to be attractive to parrots. Although sugar concentrations increased in autumn when parrot damage was greatest, seasonal differences in parrot damage were probably due to vagaries in the supply of other food sources, rather than compositional features of the phloem sap. In the driest months, concentrations of sugars were less in trees growing quickly than in those growing slowly, and greatest in trees apparently affected by water stress. Phloem sugar concentrations were therefore inconsistently related to patterns of parrot damage, and high sugar concentrations *per se* did not predispose plantation trees to a greater risk of parrot damage.

On the other hand, tree growth, sap yield and concentrations of amino acids in phloem sap were all positively related and may explain earlier results that parrot damage is greater on faster growing trees (Chapter 2). Increased concentrations of amino acids in phloem sap in autumn might also be indicative of an active cambium with high protein content, which could be of considerable nutritional benefit to parrots.

CHAPTER 5

EFFECTS OF SOIL TYPE AND CLIMATE ON CARBON ISOTOPE AND CHEMICAL COMPOSITION OF PHLOEM SAP AND FOLIAGE IN *EUCALYPTUS GLOBULUS*

5.1 Introduction

In the previous Chapter, concentrations of individual and total sugars and amino acids in phloem sap of *Eucalyptus globulus* varied greatly among plots within a plantation and, to a lesser extent, between seasons. Numerous data sets suggested availability of nutrients and water influenced these parameters but individual effects of nutrients or water were difficult to quantify.

Water stress can severely limit the growth of *E. globulus* (Dutkowski, 1995; Honeysett *et al.*, 1996; White *et al.*, 1996; Hingston *et al.*, 1998). Many trees died following the prolonged dry summer in 1993/94 due to inadequate soil moisture (Dutkowski, 1995). Mortality rates were greatest on soils of low water storage capacity, as determined by soil depth and soil type (Edwards and Harper, 1996; Hingston *et al.*, 1998; Shedley *et al.*, 1998). However, growth of *E. globulus* also depends on soil nutrient status (Cromer *et al.*, 1981; Cromer and Williams, 1982; Judd *et al.*, 1996). While the effects of water stress and nutrient status on growth in *E. globulus* are generally well known, their effects on the sugar and amino acid balance in the translocatory system have received little attention.

Continuing interest in the carbon isotope composition ($\delta^{13}C$) of leaves and wood as an integrated measure of the effect of water stress on carbon fixation (e.g. Garten and Taylor, 1992; Högberg *et al.*, 1993; Stewart *et al.*, 1995; Walcroft *et al.*, 1997) has recently been extended to eucalypts (e.g. Macfarlane and Adams 1998, Pate and Arthur 1998; Pate *et al.* 1998). According to models developed by Farquhar and others (Farquhar *et al.*, 1982; Farquhar *et al.*, 1989; Condon *et* *al.*, 1992), $\delta^{13}C$ in plant tissues (C_p) is theoretically related to the quotient of the concentration of CO_2 in intercellular spaces (C_i) and the atmosphere (C_a):

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a)\, C_i / C_a \text{ ....Equtn 5.1}$$

where $\delta^{13}C_a$ is the carbon isotope composition of the atmosphere (assumed -7.8 ‰), *a* is the fractionation of CO_2 across the stomatal pathway due to slower gaseous diffusion of the heavier $^{13}CO_2$ (4.4 ‰), and *b* is fractionation during initial carboxylation of the photosynthetic enzyme ribulose 1,5-*bis*phosphate carboxylase/oxygenase (Rubisco, ≅27 ‰). In C_3 plants, the $\delta^{13}C$ of whole plant carbon typically varies within the range -20 ‰to -35 ‰ (Ehleringer, 1989; Boutton, 1991).

Rates of both photosynthesis (A) and, indirectly, transpiration (E), affect C_i / C_a, and often both contribute to measured $\delta^{13}C_p$. Stomatal closure when water is in short supply reduces C_i and thus discrimination. Consequently, for a range of ecosystems, $\delta^{13}C$ of phloem (see Pate *et al.*, 1998), of foliage (e.g. Högberg *et al.*, 1993; Stewart *et al.* 1995) or of wood (e.g. Warren and Adams, 2000) has been used as an index of water availability. *E. globulus* responds directly to seasonal water stress by reducing transpiration through stomatal closure (Pereira *et al.*, 1986; Pereira *et al.*, 1987; White *et al.*, 1996; David *et al.*, 1997), which should reduce C_i and increase $\delta^{13}C$ due to greater enrichment and assimilation of $^{13}CO_2$ from intercellular spaces (Osório and Pereira, 1994).

The $\delta^{13}C$ of phloem sap in the main stem may better reflect shortages of water than the $\delta^{13}C$ of leaves owing to the confounding effects of age and canopy position on the latter (e.g. Garten and Taylor, 1992; Berry *et al.*, 1997; Damesin *et al.*, 1997). Carbon in phloem sap (mainly sucrose and raffinose) is largely the most recently assimilated carbon in plants, and its $\delta^{13}C$ signature should provide an almost instantaneous measure of the effects of water stress on carbon fixation (Brugnoli *et al.*, 1988). The $\delta^{13}C$ signatures of leaves or wood provide more historical, or retrospective measures of water availability.

To examine the relationship between parrot damage and tree growth further, the present study compares the effects of different soil moisture and nitrogen availabilities on total sugar and amino acid concentrations in phloem sap of young, even-aged *E. globulus*. Relationships between soil type (based on soil profile descriptions), $\delta^{13}C$, foliar nutrient concentrations and tree growth were used to identify the major factors limiting growth on contrasting sites. Soil profile descriptions remain rare in the literature but are of considerable practical value for plantation management (e.g. Turvey 1980; Turvey and Poutsma, 1980; Grant *et al.*, 1995) and offer a sound basis for distinguishing sites of varying water storage capacity and nutrient availability. Seasonal and site variations in $\delta^{13}C$ were investigated to determine the usefulness of $\delta^{13}C$ as an indicator of water stress in *E. globulus*. Some additional data are drawn from concurrent studies at the rain-fed Eulup and effluent-irrigated Albany plantations (see Pate and Arthur, 1998, and Pate *et al.*, 1998).

5.2 Methods and Materials

5.2.1 Site selection

This study consisted of three main trials, based on the 29 plots of *E. globulus* described in the previous chapter. Plots were set up in November - December, 1995 from three plantations (Klopper (K1 - K17), East (E1 - E6) and Naranbee (N1 - N6)) planted in July, 1994 in the south-west of Western Australia. Each plot consisted of ten trees (two rows x five trees). Additional data from plots at the rain-fed Eulup and effluent-irrigated Albany plantations (see Pate and Arthur, 1998 and Pate *et al.*, 1998) are also presented. Mean annual rainfall for the five plantations is Eulup - 525 mm, Klopper - 663 mm, Naranbee - 696 mm, Albany - 809 mm and East - 1432 mm. Further details of these plots can be found in Section 4.2.1, and in Pate and Arthur (1998).

Relationships among soil properties, tree growth, concentrations of nutrients in foliage and $\delta^{13}C$ were only investigated at the Klopper plantation. Relationships among tree growth, concentrations of nutrients in foliage, and $\delta^{13}C$ and total sugar and amino acid concentrations in phloem sap were assessed for all 29 plots.

A detailed study of $\delta^{13}C$ in phloem sap and foliage was conducted at two contrasting plots within the Klopper plantation (K2 and K11). $\delta^{13}C$ of phloem sap from K2 and K11 were also examined in relation to concentrations of nutrients in foliage and to patterns of gas exchange.

5.2.2 Assessment of soil type, including qualitative analysis of water storage capacity

Soil pits were dug adjacent to each plot at the Klopper plantation (except K5 and K6) to a depth of at least 1.5 m, using a backhoe. All pits were dug within two days at the end of May 1996. Although approximately 30 mm rainfall had fallen during that month, soils remained reasonably dry owing to the general lack of rain between December and April.

Soil profiles were described according to field texture, colour, depth to impeding layers, hardness, gravel content, presence of mottles, structure, and presence of fine and coarse roots in each horizon (McDonald *et al.*, 1990). Soil groups were determined from profile descriptions with reference to the soil groups of Western Australia (Schoknecht, 1997). The soil-landscape systems and sub-systems for this area have been mapped as part of the Wellington-Blackwood land resources survey, and are fully described in Tille (1996).

Samples of soil (3 sub-samples combined to a single bulk sample) were taken from each horizon immediately after the soil pit was dug, and placed in pre-weighed containers that were then sealed. The samples were re-weighed in the laboratory, and then oven-dried (60° C) for two days, and the moisture content determined on a weight basis. The average moisture content of the soil profile was calculated from the depth and moisture content of each horizon to 1.3 m. Soil moisture content was adjusted for each horizon in proportion to the amount of gravel present.

5.2.3 Analysis of soil carbon and nitrogen

Concentrations of carbon and nitrogen were determined for soil samples collected as described above and for samples of the topsoil (0 - 10 cm, and 10 - 30 cm) collected between rows in each plot at the Klopper plantation using a 10 cm soil auger. The latter samples were collected from four auger holes per plot and bulked, air dried and sieved (to 2 mm). Total carbon and nitrogen were determined by combustion in a LECO CHN analyser, calibrated with EDTA as a standard reference material. Standard reference soils and blanks were included in each run.

5.2.4 Phloem sampling

Phloem sap was collected from each plot as described previously (see Chapter 4). One bulk sample from each plot was collected in November/December 1995 ('spring') and a further sample collected in March/April 1996 ('autumn'). Samples were analysed for total sugars (sucrose + raffinose), amino acids and organic acids using HPLC, and for mineral concentration using ICP, as described in Chapter 4.

The detailed study of phloem sap composition was based on samples collected in April 1996 from the base, middle and top of the main stem from each tree at plots K2 and K11. Samples from each stem height were bulked to yield three samples per plot that were then analysed for total sugars, amino acids and $\delta^{13}C$. Further samples were collected from the mid-stem position from each tree in these two plots in May 1996 to assess between-tree variation in $\delta^{13}C$.

5.2.5 Leaf sampling

Leaves were also sampled in November/December 1995 ('spring') and March/April 1996 ('autumn') from all 29 plots. Spring samples consisted of one bulk sample (approximately 40 leaves) of young fully expanded leaves ('YFELs') per plot from the mid crown position (1.5 - 2.0 m above ground).

At this time, juvenile leaves dominated the canopy. In autumn, when tree height had increased by approximately 2.0 m, YFELs sampled from the upper crown were of the adult form.

For the detailed study, three leaf age categories (i.e. youngest leaf tips 'TIPS', YFELs, and the sixth leaf pair older than the YFEL 'YFEL + 6') and three canopy positions (base, middle and top) were sampled from each tree in plots K2 and K11 in mid-April 1996, and bulked to yield nine samples per plot. Further leaf samples (mid-crown TIPS only) were collected in early May 1996 from each tree in these two plots.

All leaf samples were oven dried at 70° C for two days and finely ground.

5.2.6 $\delta^{13}C$ analysis in phloem sap and leaves

An aliquot of undiluted phloem sap ($10\mu l$) was pipetted into tin capsules and oven dried for 15 min at 70° C, and then analysed for $^{13}C/^{12}C$ using an ANCA combustion system (Europa Instruments), coupled to a SIRA9 VG Isogas double inlet Mass Spectrometer. $\delta^{13}C$ was directly measured as the isotope natural abundance ($^{13}C/^{12}C$) in plant material (R_p) relative to that in standard reference material (R_s) i.e. fossil belemnite from the Pee Dee Formation (PDB):

$$\delta^{13}C = R_p / R_s - 1 \quad \text{...Equtn 5.2}$$

$\delta^{13}C$ values are usually negative with respect to PBD, and are expressed per mil (‰). Cornflour was used as a primary reference material ($\delta^{13}C \approx -10.78$ ‰), with other secondary standard materials included in each run. Precision of estimates of $\delta^{13}C$ between replicate samples was ± 0.1 - 0.3 ‰ (e.g. Pate and Arthur, 1998). Due to the high level of precision, only every tenth sample was duplicated.

Oven dried foliage was finely ground in a ball mill, and re-dried at 70° C for 15 min. $\delta^{13}C$ analysis of ground foliage samples was as described for phloem samples.

5.2.7 Analysis of nutrient concentrations in foliage

Oven dried leaves were finely ground and approximately 0.1 g accurately weighed and digested in 3 ml c.H_2SO_4 with salicylic acid and H_2O_2 added (Fleck, 1974; Dalal *et al.*, 1984). Total nitrogen in the digest was determined colorimetrically using a modified Berthelot reaction (Searle, 1984). Phosphorus was also determined colorimetrically using ammonium molybdate, potassium antimony tartrate and ascorbic acid (after Murphy and Riley, 1962), while potassium was determined using atomic absorption spectrometry (AAS) with caesium as an ionisation suppressant. All samples were digested and analysed in duplicate, and each digestion batch included standard reference materials and blanks.

5.2.8 Tree growth

Diameter at breast height (DBH) of each tree in each plot was measured in November/December 1995, and again in March/April 1996. Plots in the Klopper plantation were also measured periodically over the next two years, with a final measurement in April 1998. A number of trees had died within this plantation, including some in experimental plots, in the summer of 1997/8.

5.2.9 Gas exchange measurements

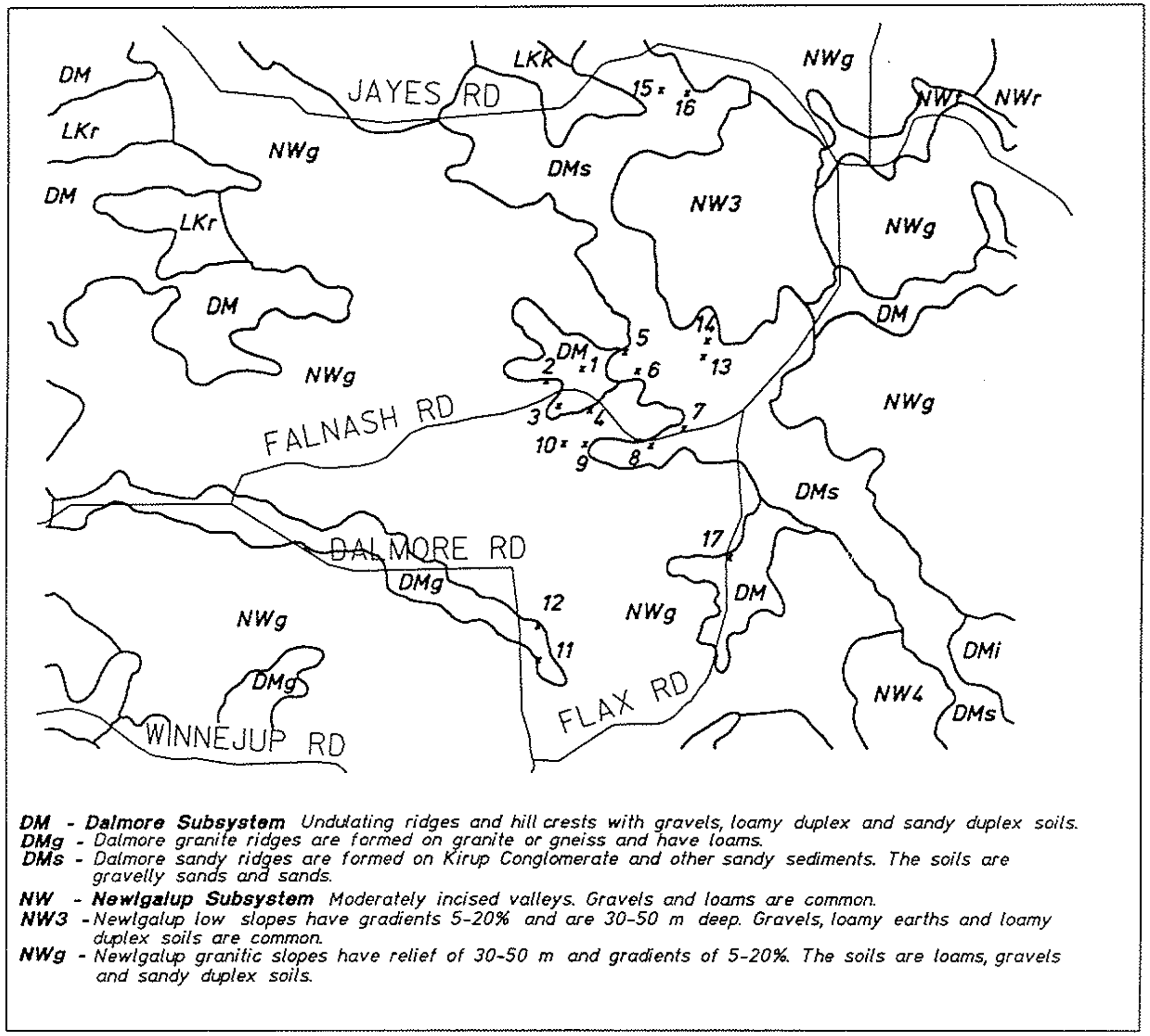
Leaf gas exchange was measured on all ten trees in two plots in the Klopper plantation, K2 and K11, using an open infra-red gas analyser (LCA-4, Analytical Development Co., Hoddesdon, UK) attached to a Parkinson leaf chamber (was 6.25 cm^{-2} area). Airflow through the chamber was 250 $\mu mol\ s^{-1}$. Two unshaded, attached, fully expanded juvenile leaves (YFEL +1) with a northerly aspect were selected per tree (i.e. 20 leaves per plot) from the lower to mid-canopy (1.5 - 2.0 m above ground). Net photosynthesis (A), transpiration (E), stomatal conductance (Gs), photosynthetically active photon flux density (PAR), leaf surface temperature (T_l), and atmospheric (C_a) and internal (C_i) CO_2 partial pressures were measured over four time periods (approximately 45 - 60 min per plot) on a single day in early May 1996. The same parameters were characterised for six adult leaves (one leaf pair older than YFEL,'YFEL+6') per plot and there were no appreciable differences between juvenile and adult leaves.

On the day of measurement, the sky was overcast until approximately 1130 h, and remained cloudy for most of the day. Air temperatures varied from a minimum of 7.0° C to a maximum of 24.5° C at approximately 1430 h. Plots were measured consecutively and so comparisons between plots at each time period are indicative. Statistical differences between plots were determined by comparing the mean values of all measurements recorded, for each gas exchange characteristic, over the four time periods (n = 80).

5.3 Results

5.3.1 Soil profile descriptions

The Klopper plantation lies in the Eastern Darling Range Zone (Tille 1996). The terrain is dissected and undulating and contains remnants of the deep lateritic plateau formed on granite and gneiss. Two systems within this zone are represented in this plantation, the Eulin Uplands system and the Boyup Brook Valleys system. The Eulin Uplands system is dominated by broad ridges formed on laterite, granite and Kirup Conglomerate (Dalmore subsystem). The Boyup Brook Valleys system occurs where the Blackwood River dissected the Eulin Uplands system to form moderately incised valleys through granitic and gneissic terrain (Newlgalup subsystem, Fig. 5.1, Tille, 1996).



DM - **Dalmore Subsystem** *Undulating ridges and hill crests with gravels, loamy duplex and sandy duplex soils.*
DMg - *Dalmore granite ridges are formed on granite or gneiss and have loams.*
DMs - *Dalmore sandy ridges are formed on Kirup Conglomerate and other sandy sediments. The soils are gravelly sands and sands.*
NW - **Newlgalup Subsystem** *Moderately incised valleys. Gravels and loams are common.*
NW3 - *Newlgalup low slopes have gradients 5-20% and are 30-50 m deep. Gravels, loamy earths and loamy duplex soils are common.*
NWg - *Newlgalup granitic slopes have relief of 30-50 m and gradients of 5-20%. The soils are loams, gravels and sandy duplex soils.*

Figure 5.1 Map of land systems in the study area, adapted from Tille (1996), showing the location of each plot (K1 -- K17) in the Klopper plantation.

Figure 5.2 Red brown loamy duplex soil over dolerite at a site (K10) in the Klopper plantation showing large columnar structure, and the effect of shrinking and swelling of clay on root growth.

The soils in this area vary greatly, from deep, bleached sandy gravels formed on ironstone or conglomerate along laterite ridges, to young, usually shallow, red-brown loams formed directly on granite or gneiss along incised valleys (Tille, 1996). Soils vary in colour, soil depth, depth to clay, clay and gravel content, and presence of impeding layers (e.g. ironstone, heavy clay or silcrete).

Soil profiles were classed into 5 soil groups (after Northcote, 1979) with descriptions as follows:

1. Red-Brown loamy gravel duplex (Dr4.12; plots K2, K9, K10, K12 and K17). Reddish-brown loamy topsoil with 20 – 80 % ironstone gravel and fragments over a reddish clay. At K2, K12 and K17, the topsoil was shallow (to 10 cm) and the clay subsoil more gradational, than in K9 and K10 (topsoil to 40 cm). The loamy topsoil tended to be massive with large blocky peds (>10 cm), and was very dry with few fine roots. At plot K10, coarse roots were confined to the major cracks between the large blocky peds, and were visibly 'squashed' suggesting repeated wetting and drying of the peds (Fig 5.2). The clay was medium - heavy at K9, K10 and K12 and appeared to restrict root development, while at K2 was light to medium and well structured with abundant and evenly distributed roots. All plots had yellow and orange mottles at 40 - 60 cm, and some partly weathered rock fragments at 90 - 120 cm. Saprolite (soft weathered rock) was present in K10 at 90 cm, and in K12 at 120 cm. White dispersive clay appeared at 90 cm in K17, with no apparent restriction to root development to 170 cm.

2. Shallow red-brown loam (Gn2.12; plot K11). A shallow profile consisting of red-brown loamy topsoil with massive blocky structure, over an abrupt boundary to yellow-brown clayey sand with partially weathered granitic saprolite at 30 - 140cm (Fig.5.3). Beneath the saprolite, heavy red-grey mottled clay overlayed rock at 150 cm. The structureless saprolite was hard and dry, and few roots were observed below 50 cm.

Figure 5.3 Shallow red brown loam over structureless saprolite (30 – 140 cm), over granite (K11) in the Klopper plantation. Many young plantation trees died of drought in April 1998 on this shallow soil due to its very low water holding capacity.

3. Brown loamy duplex (Dy3.12, plot K3). A soft brown loam to sandy loam topsoil graded to slightly cemented yellow clay sand at 50 cm. A clear boundary overlay slightly cemented, grey, medium clay with red and orange mottles at 70 cm, with hard, cemented, medium clay and saprolite at 100 cm. This profile was shallow and granitic rock was present at 130 cm. There were few fine or coarse roots below 50 cm.

4. Yellow-brown loamy gravel duplex (Dy5.12; plots K1, K4 and K14). A topsoil of soft, yellow-brown loam to sandy loam or clayey sand with 10 - 60 % rounded ironstone gravel, over structured, sandy, light clay at 40 cm (70 cm for K14) with no gravel, and with many fine and coarse roots. A gradational change to slightly cemented, yellow-orange light clay with red and white mottles to 140 cm, which tended to be hard and massive with few fine roots. Coarse roots were present at this depth, but were mainly confined to old root channels.

5. Moderately deep sandy gravel. These profiles all consist of moderately deep sandy gravels over clay at 30 - 80 cm. This group was further divided into:

5a. Pale moderately deep sandy gravel (Dy5.4; plots K7, K8 and K15). Topsoils were grey, loamy, sands with 5 - 10 % gravel, over bleached sand or sandy loam with 50 - 80 % irregular gravel from 30 - 70 cm (50 - 90 cm for K15). A clear boundary lead to yellow sandy clay, which was slightly cemented, massive, hard and mottled to a depth of 130 cm. At K7 and K8, this horizon also contained 50 - 60 % partly cemented, ironstone gravel and quartzose cobbles up to 130 mm (Kirup Conglomerate), whereas at K15, this horizon had no gravel and the sandy clay was massive and porous, but not cemented. Beneath this horizon was gritty sandy medium clay, with slight mottles. Root development was restricted through the heavy ironstone gravel in soil pits 7 and 8, but some coarse roots were present at 150 cm in soil pit 8. Soil pit 15 had prolific fine root development in the top 90 cm, with some fine and coarse roots present to 150 cm.

5b. Shallow gravel (Uc5.11; K13 and K16). Similar to the above group, but cemented ironstone caprock was present at 50 - 90 cm (K13) and 70 - 130 cm (K16). Beneath this lay moderately cemented, yellow sandy clay with distinct orange, red and white mottles. Roots were common in the topsoil, but were severely restricted by the ironstone layer. Even so, the caprock was not continuous, and some coarse roots exploited old root channels through the caprock and extended into the clay.

In general, soil texture, structure, and nature of impeding layers were major factors in the distribution and abundance of both fine and coarse roots at the Klopper plantation. Fine roots were abundant in soft sandy or sandy loam topsoils, but were scarce where the topsoil was massive with large blocky peds (e.g. K10 and K12) and largely confined to cracks between large columnar peds. Coarse roots were severely restricted by the presence of heavy gravel and lateritic caprock, and by medium to heavy clay horizons. The clayey B horizon of many duplex gravel soils was moderately cemented with little structure. These impeding layers often limited the vertical growth of roots to old root channels.

The cemented B horizon not only limited root growth, but contributed to waterlogging during the wet winter months. Three days after 140 mm rain in June, only four soil pits had drained completely (i.e. K2, K4, K8 and K12). Pits at K3 and K10 remained two-thirds full of water. The presence of strong mottles and bleached A2 horizons was further evidence that many soils were prone to seasonal waterlogging.

5.3.2 Tree growth in relation to nutrient and moisture availability at the Klopper plantation

Concentrations of total carbon ranged from 2.5 - 10.7 % and of total nitrogen from 0.14 - 0.64 %. Total N was strongly and linearly related to total C (r^2 = 0.84, $P<0.001$, Fig 5.4) with an overall mean C:N ratio of about 14. In individual plots, C:N ratios varied from 12 - 23. Mean total N decreased significantly with depth ($P<0.001$) and was typically less than 0.03 % at a depth of 1.3 m (results not presented). Up to 60 % of total N was present in the top 0 - 10 cm.

Grouping soil profiles broadly into either red loams or sandy gravel showed that C:N ratios were less, and moisture contents greater, in 0 – 10 cm soil samples in red loams, but these parameters were unrelated to tree growth (Table 5.1). Weighted soil moisture contents for whole profiles (0 - 130 cm) were also greater in red loams (13.1 %) than sandy gravels (4.7 %, $P<0.01$) (Fig. 5.5), probably due to greater matric potentials in the former and hence greater retention of water at low soil water potentials (Rawls *et al.*, 1982).

Differences in growth over time for representative soil types are shown in Fig. 5.6. Mean basal area per tree was consistently greatest in K2 and least in K11, where a majority of trees died immediately before the final measurement due to limited soil depth and inadequate soil moisture. Clearly, trees growing on the deeper soils (> 2 m soil depth) with no impediments to root growth (i.e. K1, K2, K4, K14 and K15) were able to access moisture at depths greater than 130 cm. Soil depth has a much greater effect on soil water storage capacity than soil type. Trees that were most prone to water stress were those growing in the shallowest soils, i.e. K3 and K11, where rock was present at 130 cm and 150 cm, respectively. Soils at K10 and K12 would also have had limited total water storage, given the presence of saprolite at 90 cm and 120 cm. Differences in rates of growth were clear before trees reached two years of age (Fig. 5.6) and were maintained over the 3 years of measurement.

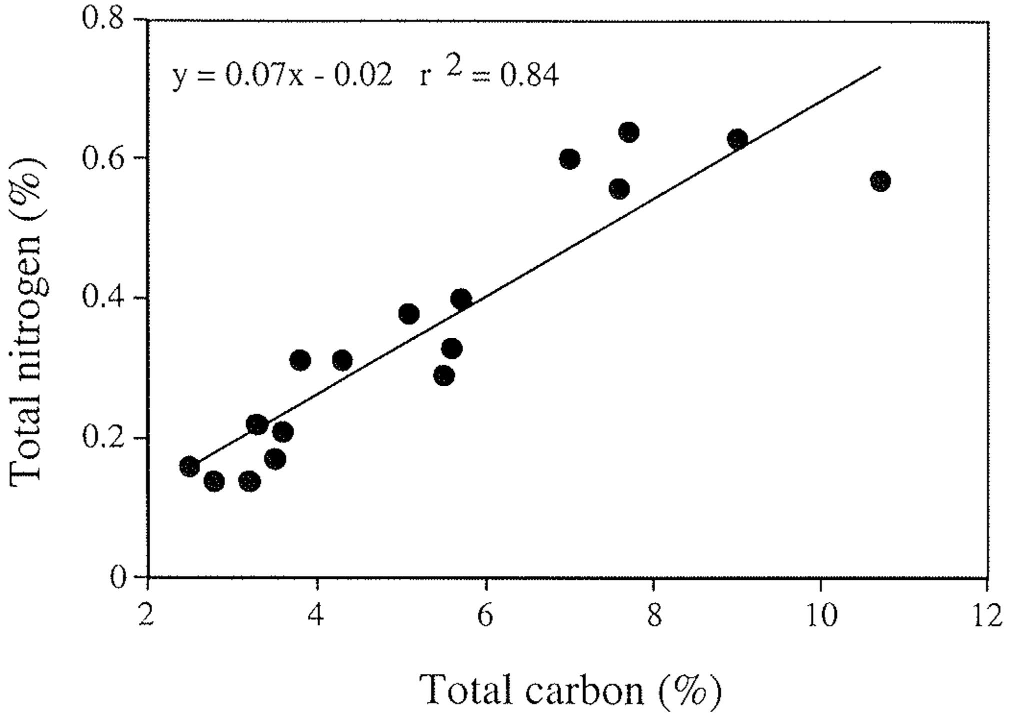


Figure 5.4 Total carbon and nitrogen concentrations in soil from the Klopper plantation sampled from the top 0 – 10 cm of each plot in April 1996.

Table 5.1

Mean concentrations of total carbon and nitrogen (0-10 cm) and soil moisture content (0 - 30 cm) for soil profiles with either red loam (n = 6) or sandy gravel (n = 11) topsoils. Mean diameter at breast height of trees (DBH) is shown. Significance of differences between means for each soil type from one-way ANOVA are shown ****P*<0.001, ***P*<0.01, **P*<0.05.

	Sandy gravel	Red loam	*P*
Total C (%)	4.8	6.3	ns
Total N (%)	0.30	0.46	ns
C:N	17.1	13.8	*
Moisture content (%)	5.86	11.48	*
DBH (cm)	5.45	4.86	ns

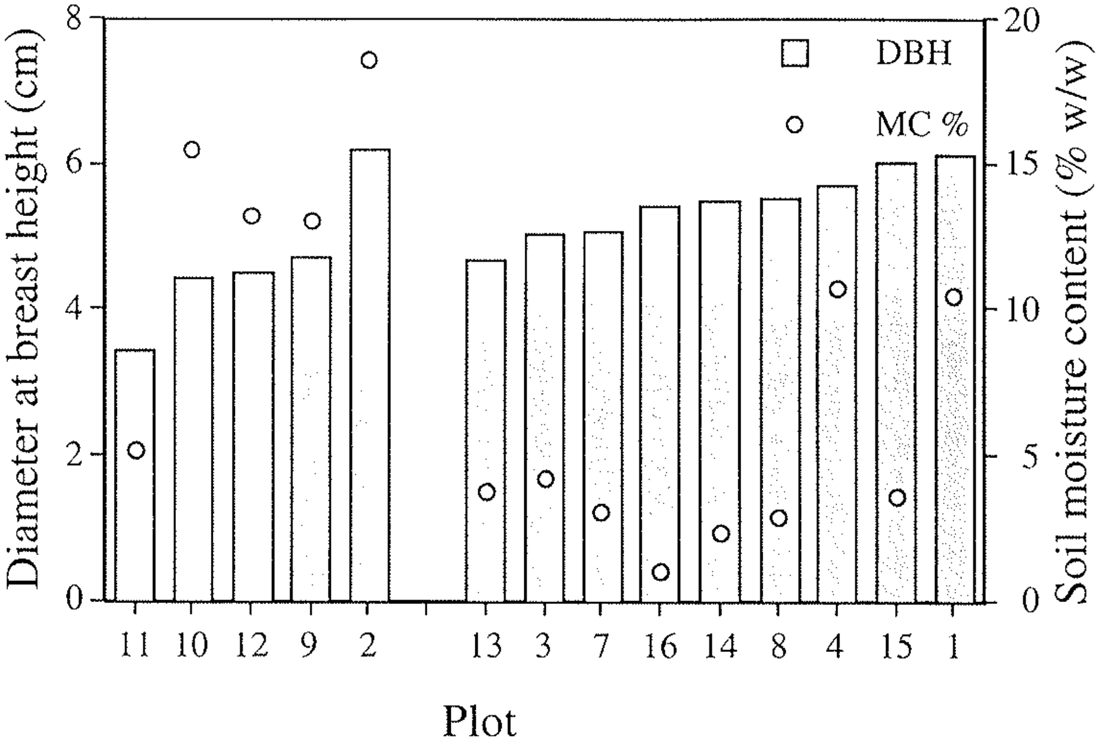


Figure 5.5 Mean diameter of trees in plots at the Klopper plantation and soil moisture content (%, oven dry basis, weighted mean) of profile to 130 cm. Red loams or red loamy gravels are to the left, yellow or bleached sandy gravels are to the right.

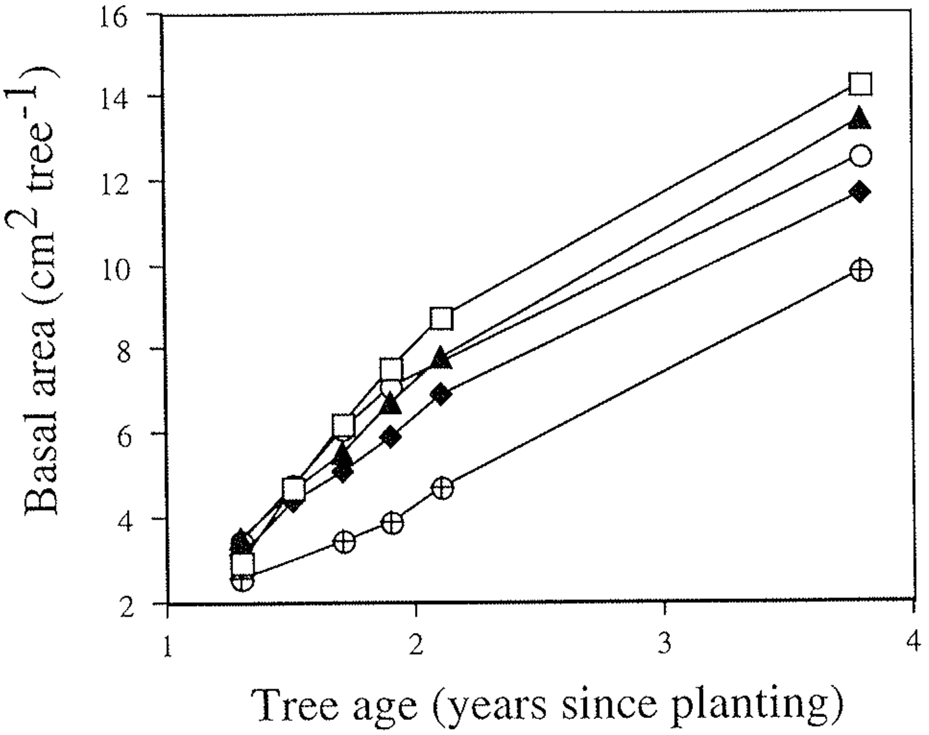


Figure 5.6 Change in basal area for representative plots at the Klopper plantation between November 1995 and April 1998. Plots were selected to cover the range of recorded basal areas. About 70 % of trees in plot K11 died from drought just prior to the final measurement – the diameters of these trees were included.

5.3.3 Seasonal and spatial variation in phloem $\delta^{13}C$ values

At the Klopper plantation, mean phloem $\delta^{13}C$ values were significantly greater in autumn (-22.6 ‰) than in late spring (-24.4 ‰) ($P<0.001$, n = 16). Phloem $\delta^{13}C$ ranged from -26.7 ‰ to -22.3 ‰ in spring, and from -25.3 ‰ to -20.2 ‰ in autumn (Fig. 5.7). A similar range was found in autumn from the other low rainfall plantation, Naranbee (-23.8 ‰ to -20.4 ‰). By comparison, at the East plantation in the high rainfall region, $\delta^{13}C$ values were always less than -24.6 ‰.

Large seasonal differences in $\delta^{13}C$ at the Klopper plantation were recorded for plots K3 (-25.2 ‰ to -20.6 ‰) and K11 (-25.4 ‰ to -20.2 ‰, Fig. 5.7), both of which had shallow soils (see soil profile descriptions). Autumn $\delta^{13}C$ values from other plots with either shallow soils (K10 and K12) or soils with significant impediments to root growth (K7, K8 and K13) lay between -21.0 ‰ and -22.0 ‰. In contrast, plots K1 and K2 had deep soils with no impeding layers, greatest mean tree diameters (Fig. 5.5) among all plots, and phloem $\delta^{13}C$ values that were less in autumn than in spring (Fig.5.7). Not surprisingly, the large differences in $\delta^{13}C$ among plots at the Klopper plantation were strongly correlated with mean tree diameter ($r^2 = 0.678$, $P<0.001$, Fig. 5.8) when measured in autumn. There was no relationship when measured in spring. When re-measured two years later, in April 1998, diameter was still strongly related to the $\delta^{13}C$ of phloem sap ($r^2 = 0.698$, $P<0.001$). Mean basal area at that time for the most stressed plot, K11, was 75.4 cm^2 $tree^{-1}$ compared with 158.4 cm^2 $tree^{-1}$ at K2. Thus high phloem $\delta^{13}C$ values (>-23.0 ‰) were consistently associated with low soil water availability, and suggests that many trees in the low rainfall region were affected by low soil water availability, some (e.g. K10 and K13) as early as November.

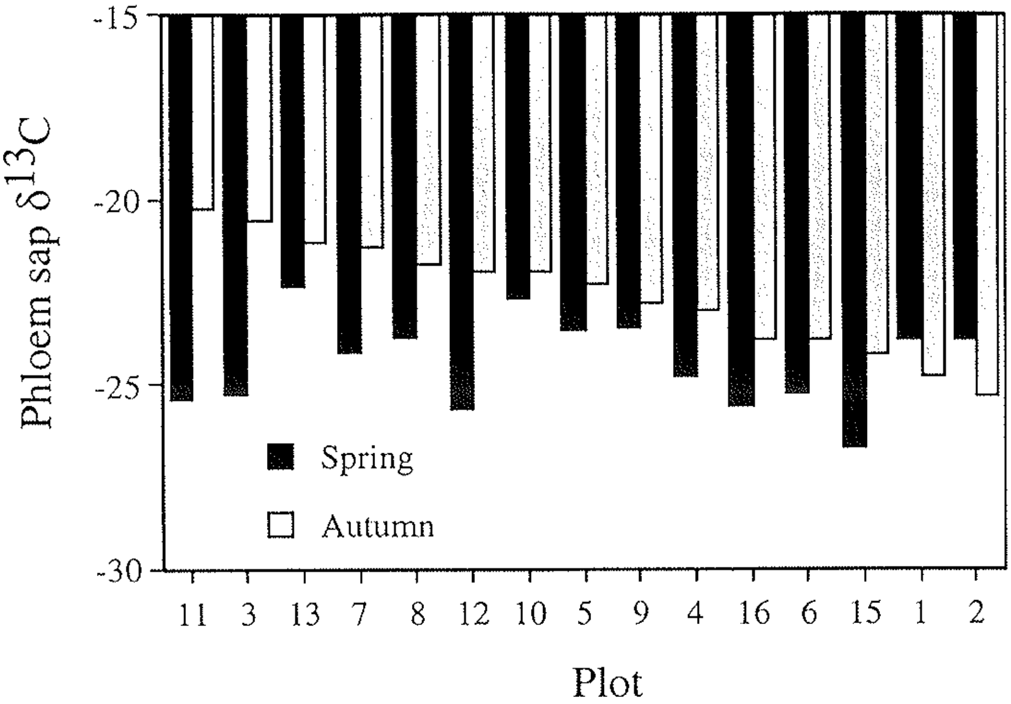


Figure 5.7 Seasonal variation in phloem $\delta^{13}C$ for all plots (except K14) at the Klopper plantation. Phloem was collected as one bulk sample per plot in November 1995 (Spring) and in March 1996 (Autumn).

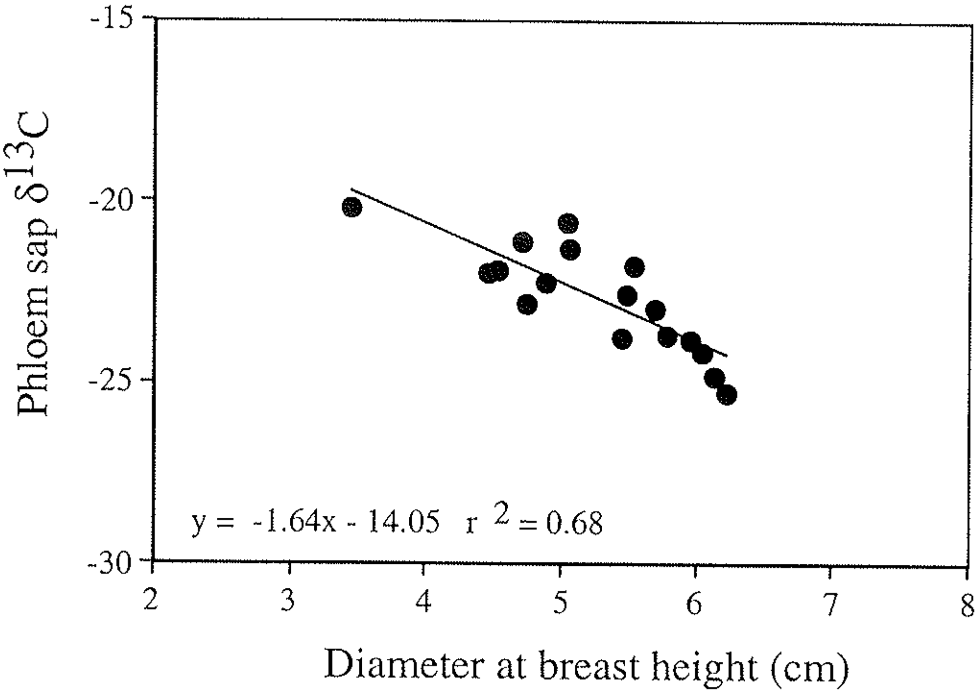


Figure 5.8 Relationship between diameter at breast height (DBH) and δ^{13}C in the phloem sap for all plots at the Klopper plantation, in autumn 1996.

5.3.4 Case study of two contrasting plots at the Klopper plantation

A more detailed study of plots K11 (shallow soil over rock) and K2 (deep, well structured soil) in April 1996 contrasted phloem and foliage $\delta^{13}C$ (Table 5.2). Mean foliar $\delta^{13}C$ values at K11 (-23.7 ‰) were significantly ($P<0.01$) greater than at K2 (-25.9 ‰). In general, at each sampling height $\delta^{13}C$ of foliage was greatest in newly developing leaf tips and least in the oldest leaves sampled, and phloem $\delta^{13}C$ values were consistently greater than corresponding foliar values (Table 5.2). Phloem and foliage $\delta^{13}C$ values also increased with stem height (Table 5.2). Leaf TIPs from the mid-canopy, sampled from each tree a fortnight later (2nd May), had even greater $\delta^{13}C$ values at K11 (-20.0 ± 0.3 ‰) than in April (-22.0 ‰), while those from K2 showed little change (-25.1 ± 0.3 ‰ c.f. -25.5 ‰, respectively).

In the same plots, patterns of gas exchange were largely dictated by the interaction of available light and water (Table 5.3). Photosynthetically active radiation (PAR) was about 50 % less at K2 (mean DBH 6.9 cm) than at K11 (mean DBH 3.6 cm) yet mean rates of net photosynthesis and transpiration at K2 were almost twice those at K11, and stomatal conductance was more than 3-fold greater at K2. Stomatal conductance was not only greater at K2, but remained greater for longer periods during the day (results not presented). C_i did not vary significantly between plots ($n = 80$) suggesting that reduced stomatal conductance at K11 was balanced by a proportionately lower rate of net photosynthesis.

Mean C_i/C_a ratios for K11 were used to calculate foliar $\delta^{13}C$ (see Introduction). Differences in C_i/C_a translated into $\delta^{13}C$ in foliage of -23.3 ‰ for K11 and -25.1 ‰ for K2 (Table 5.3). These values are remarkably similar to those measured in mid-canopy leaf tips sampled at the same time (i.e. K11 -22.0 ‰ and K2 -25.5 ‰, Table 5.2).

Table 5.2
Carbon isotope ratios ($\delta^{13}C$) of phloem sap collected from the top, middle and base of the main stem from trees growing in plots with contrasting soil profiles at the Klopper plantation. Corresponding $\delta^{13}C$ of three age classes of foliage (i.e. TIPS, YFELs, YFELs + 6) sampled at the same time (April 1996) from the top, middle and base of the canopy are also shown.

Plot	Position	Phloem $\delta^{13}C$	YFEL +6 (‰)	YFEL	TIP
K2	Top	-22.0	-26.6	-25.5	-23.2
	Middle	-22.4	-26.5	-26.8	-25.5
	Base	-23.1	-26.9	-26.6	-25.8
K11	Top	-18.8	-24.1	-22.8	-21.2
	Middle	-18.8	-25.8	-24.1	-22.0
	Base	-19.6	-26.4	-24.5	-22.6

Table 5.3
Summary of gas exchange characteristics, measured on 3rd May 1996, for K2 and K11. Data were analysed using one-way ANOVA (n = 80). Significant differences between means are shown by *** ($P<0.001$), ** ($P<0.01$), * ($P<0.05$), ns (not significant).

Parameter	Plot K2	 K11	*P*
A μmol m^{-2} s^{-1}	3.75	1.97	***
E mmol m^{-2} s^{-1}	0.662	0.380	***
Gs mol m^{-2} s^{-1}	0.065	0.020	***
PAR μmol m^{-2} s^{-1}	191.8	305.6	***
T_l °C	20.4	22.6	*
Ci vpm	264.2	254.2	ns
C_i / C_a	0.72	0.67	0.056
Calculated δ^{13}C (‰)	-25.2	-23.2	0.056
A/E mmol mol^{-1}	4.39	4.05	ns

Foliar N concentrations increased with canopy height and decreased with leaf age, and were significantly greater in trees at K2 (1.87 %) than at K11 (1.43 %) ($P<0.001$). However, there was no significant relationship between foliar N and foliar $\delta^{13}C$ values for all samples.

5.3.5 Relationships between tree growth, phloem $\delta^{13}C$ and foliar nutrient concentrations

A subset of the four plots at the Klopper plantation (K1, K2, K3 and K11), were contrasted with four plots from each of the Naranbee and East plantations. Concentrations of N, P or K in foliage were not significantly related to DBH in the plots at the Klopper plantation. Trees with the greatest phloem $\delta^{13}C$ values and least DBH (K3 and K11) had foliar concentrations of N, P and K equal to, or greater than those in the best growth plots (Table 5.4). At the high mean annual rainfall East plantation, variability in diameter was not related to phloem $\delta^{13}C$, but poor growth in plots E3 and E6 was variably associated with deficient concentrations of N, P and K in foliage (Table 5.4). At the Naranbee plantation, poorest growth was associated with deficient concentrations of N, P and K (N5) as well as water stress (N6). Across all plots, diameter was weakly related to foliar concentrations of nitrogen ($r^2 = 0.294$, $P<0.01$), potassium ($r^2 = 0.305$, $P<0.01$) and phosphorus ($r^2 = 0.317$, $P<0.01$), and inversely related to phloem $\delta^{13}C$ ($r^2 = 0.169$, $P<0.05$), but there was no relationship between phloem $\delta^{13}C$ and any of the foliar nutrients measured. When these factors were combined in a multiple regression they accounted for approximately 62 % ($P<0.001$) of the variation in DBH in autumn.

Table 5.4

Autumn data for $\delta^{13}C$ in phloem, and concentrations of major nutrients in youngest fully expanded leaves, from a subset of plots from the Klopper (K), Naranbee (N) and East (E) plantations. Concentrations in the marginal range for nutrients are marked *, and those in the deficiency range are marked ** (*sensu* Reuter and Robinson, 1997). Plots were selected to cover the range of best and worst growth at each plantation.

Plot	DBH (cm)	$\delta^{13}C$ (‰)	N %	K %	P %
K2	6.21	-25.3	1.60*	0.59*	0.10*
K1	6.13	-24.8	1.79	0.84	0.12
K3	5.03	-20.6	1.67*	0.90	0.11*
K11	3.45	-20.2	1.65*	0.99	0.10*
N3	7.53	-23.5	2.40	1.01	0.18
N2	6.58	-23.0	1.90	0.77*	0.12
N6	3.76	-20.4	1.33*	0.31**	0.08**
N5	3.30	-23.8	0.96**	0.39**	0.07**
E4	8.12	-24.6	1.73	0.82	0.11*
E2	7.36	-25.7	1.86	0.98	0.13
E6	3.60	-24.7	1.68*	0.23**	0.09**
E3	3.13	-25.9	1.37*	0.15**	0.11

5.3.6 Effect of water stress and nutrient availability on composition of phloem

(i) Total sugars

Phloem $\delta^{13}C$ values in autumn were strongly related to total sugar concentrations ($r^2 = 0.70$, $P<0.001$) in phloem sap collected from all plantations (Fig. 5.9). Including data from recently published studies (Pate and Arthur, 1998) at the rain-fed Eulup plantation and the effluent-irrigated Albany plantation, phloem $\delta^{13}C$ values in autumn ranged widely from -19.3 ‰ to -27.2 ‰, while total sugar concentrations varied in parallel from 31.4 % (w/v) to 20.7 % (w/v). A similarly wide range of phloem $\delta^{13}C$ and total sugar concentrations found in this study among all plots in autumn was found at the Eulup plantation on a seasonal basis (see Pate *et al.*, 1998). As there were no significant relationships between sugar concentrations and any of the foliar nutrients analysed, variation in sugar concentrations are assumed to be primarily associated with differences in soil water availability.

(ii) Total amino acids

Concentrations of total amino acids in phloem sap (>98 % of total N) varied considerably at the Klopper, Naranbee and East plantations (overall range 0.7 - 20.2 μmol ml^{-1}). Concentrations of amino acids were weakly related to phloem $\delta^{13}C$ ($r^2 = 0.145$, $P<0.05$), but were strongly related to foliar N concentrations ($r^2 = 0.41$, $P<0.001$, Fig. 5.10). These two factors together explained 58 % ($P<0.001$) of the variation in the concentration of amino acids in phloem for all plots:

$[A] = -42.8 + 14.2[N] - 1.26\,\delta^{13}C$ ....Equtn 5.3

where [A] is the concentration of amino acids (μmol ml^{-1}) in phloem sap, and [N] is the concentration of total nitrogen (%) in young, fully expanded leaves (YFELs). At the Klopper plantation (n = 16), the multiple regression equation explained 68 % ($P<0.001$) of the variation in phloem amino acid concentrations. Likely variation in amino acid concentrations with variation in foliage N and phloem $\delta^{13}C$ are shown in Fig. 5.11. Clearly, large differences in amino acid concentrations in phloem may result either from variation in foliar N, or could be found among plots with similar foliar N, due to differences in the availability of water.

If soil nitrogen is included as a further factor in the regression analysis for the Klopper plantation, along with foliar nitrogen and phloem $\delta^{13}C$, some 72 % of the variation in total amino acid concentrations could be predicted ($P<0.01$):

$$[A] = -71.3 + 19.2[N_l] + 7.3[N_s] - 2.1\delta^{13}C \text{........................Equtn 5.4}$$

where [A] is the concentration of total amino acids in phloem sap (μmol ml^{-1}), $[N_l]$ is the total concentration of nitrogen (%) in YFELs, and $[N_s]$ is the total concentration of nitrogen (%) in soil (0 - 10 cm).

(iii) Potassium

In autumn, potassium concentrations in YFELs in the upper crown were also closely related to the concentration of potassium in phloem sap ($r^2 = 0.77$, $P<0.001$) for all plots (Fig. 5.12). Since growth of *E. globulus* was related to potassium concentrations in YFELs and in phloem sap (see Chapter 4), it may be possible to use phloem sap analysis for diagnosis of potassium deficiency.

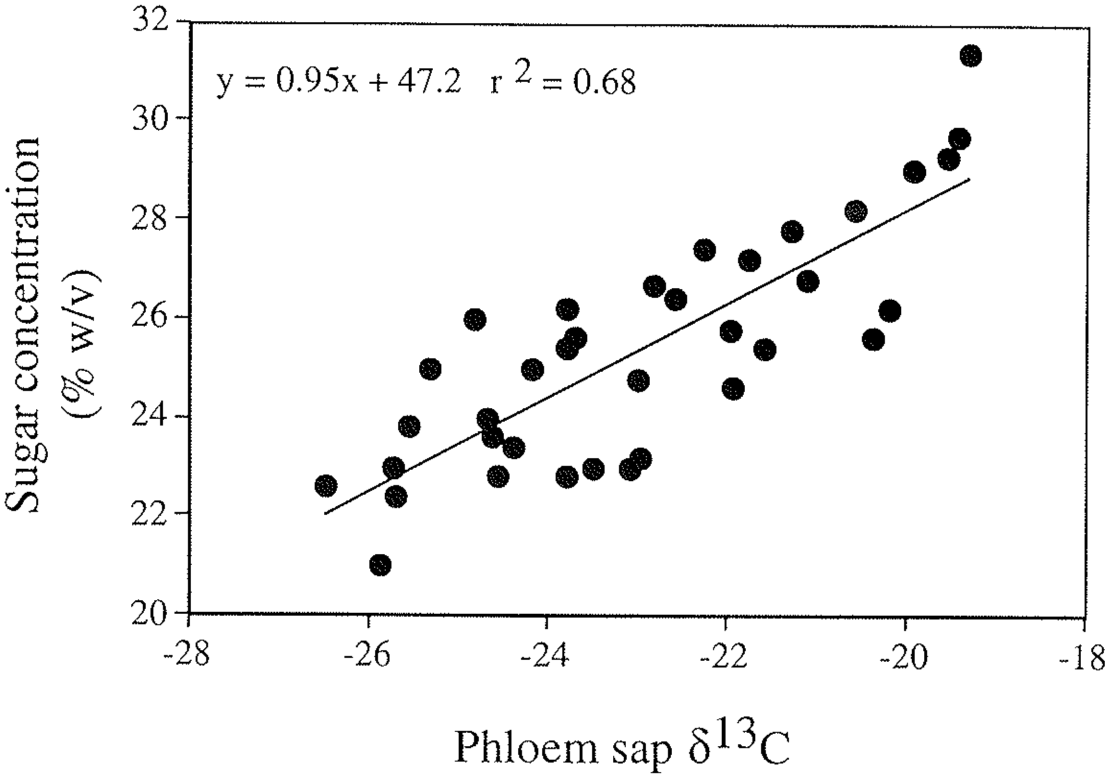


Figure 5.9 Relationship between total sugar concentrations and $\delta^{13}C$ in phloem sap from even-aged *E. globulus*, collected in autumn. Samples were taken from 33 plots in the Klopper, Naranbee, East, Eulup and Albany plantations, including data from Pate and Arthur (1998) and Pate *et al.* (1998) (with permission).

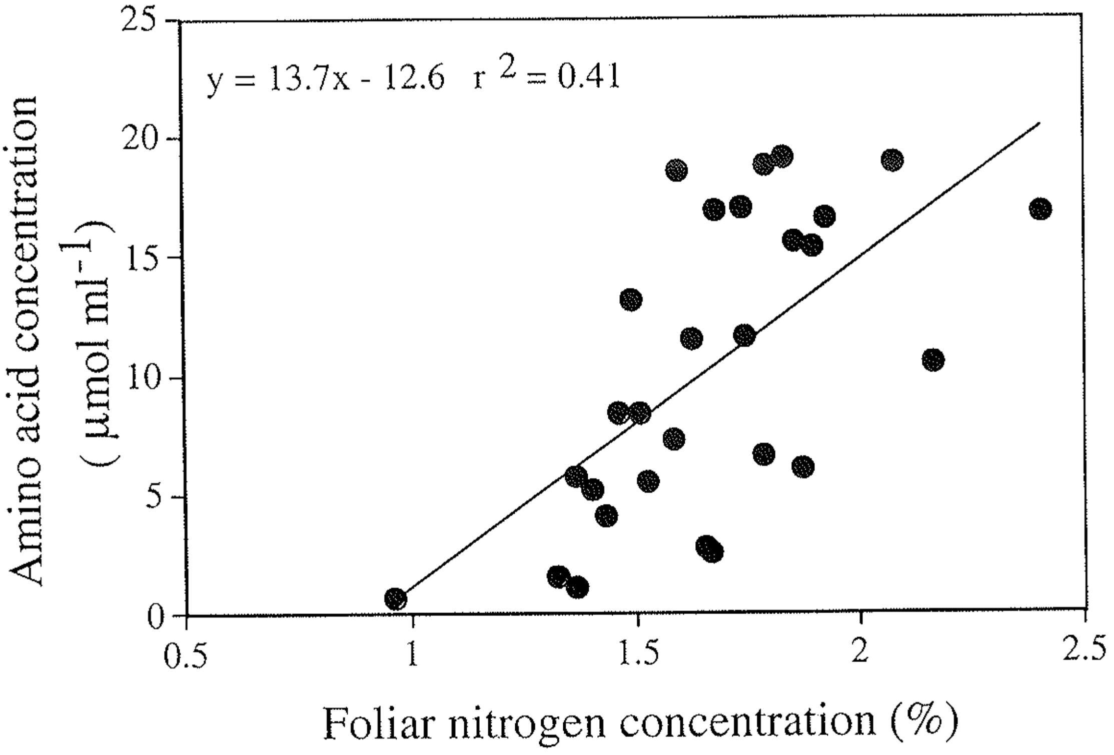


Figure 5.10 Relationship between nitrogen concentrations in young fully expanded leaves (YFELs) from the upper crown, and total amino acid concentrations in phloem sap from stems of *E. globulus*, for all plots in the Klopper, East and Naranbee plantations in autumn.

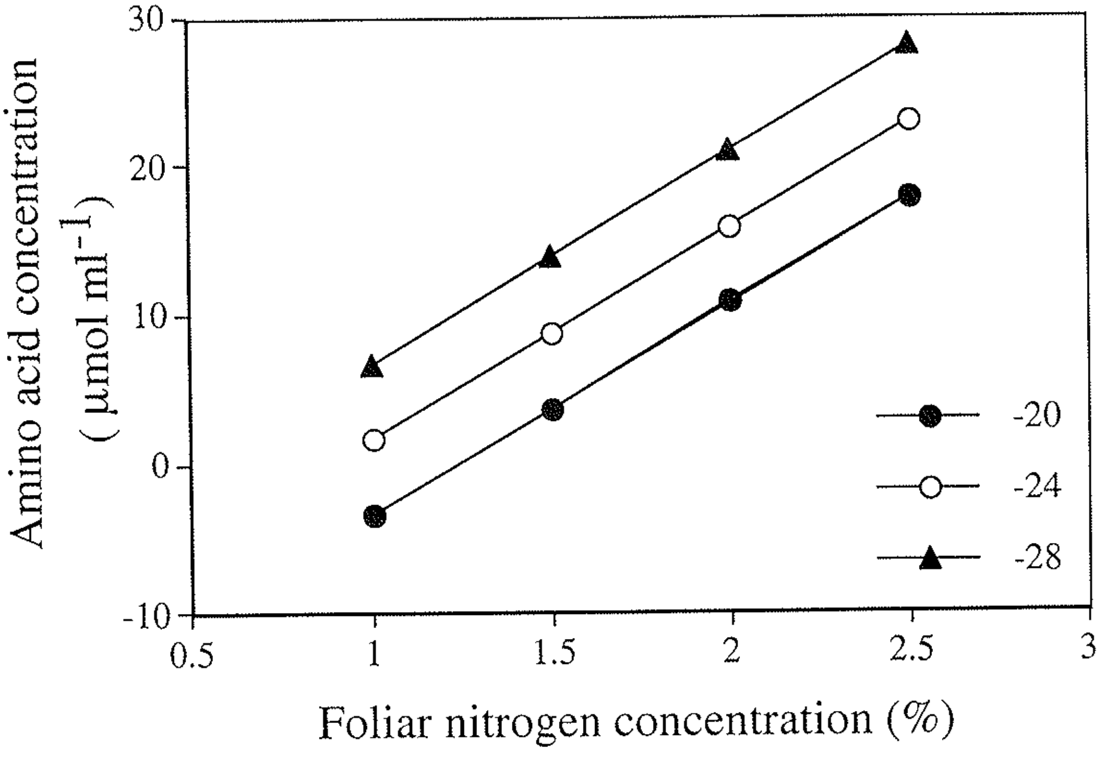


Figure 5.11 Predicted relationships between total amino acid concentrations in phloem sap and foliar nitrogen concentrations in youngest fully expanded leaves (YFELs) with a range of likely phloem $\delta^{13}C$ values (see text for details). Selected values of $\delta^{13}C$ are: -20.0 ‰, -24.0 ‰ and -28.0 ‰.

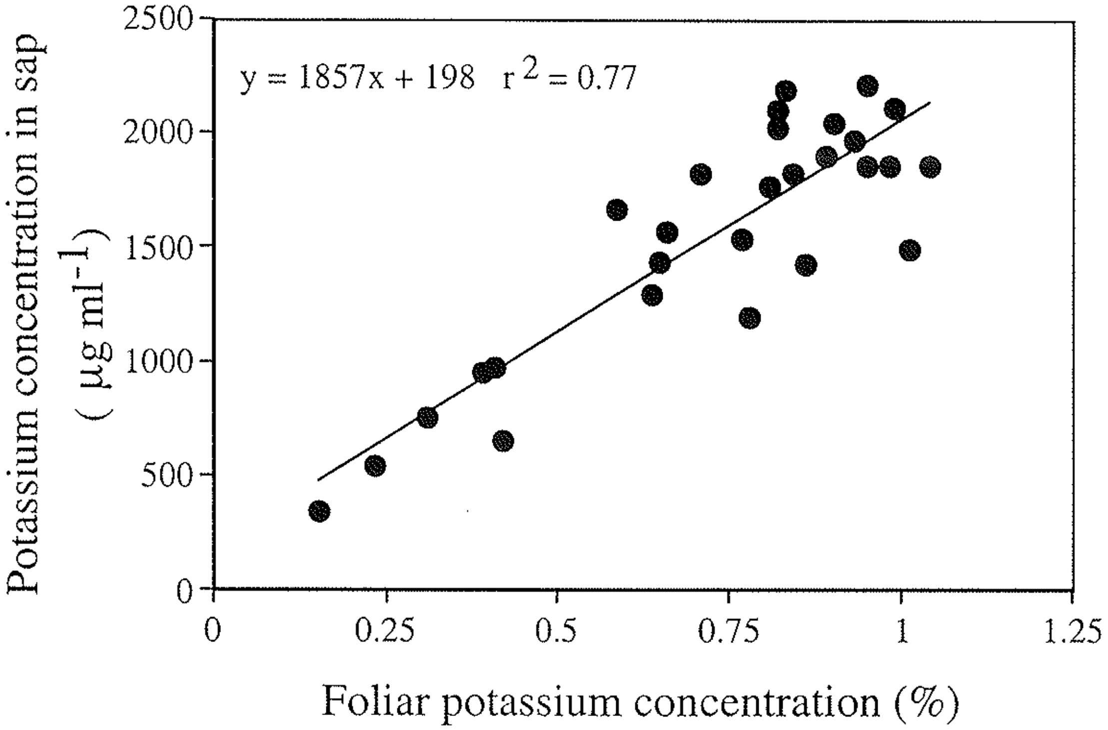


Figure 5.12 Relationship between potassium concentrations in young fully expanded leaves (YFELs) from the upper crown and potassium concentrations in phloem sap collected from all plots at the Klopper, Naranbee and East plantations in autumn.

5.4 Discussion

$\delta^{13}C$ signatures of phloem sap collected from the mid-stem of even-aged plantation-grown *E. globulus* clearly reflected differences in soil moisture availability. Studies within the Klopper plantation highlighted large variations in soil profiles, which markedly affected tree growth and phloem composition, often within short distances (<100 m). Shallow soils with bedrock at ~ 1.5 m or where soil graded to saprolite between 1 – 2 m, had limited water storage capacity and correspondingly large $\delta^{13}C$ in autumn (>21 ‰), at the end of the hot dry summers characteristic of the region. Profiles with soil depths greater than 2 m and without serious impediments to root growth produced good tree growth and lower $\delta^{13}C$ values (< -24 ‰). Thus, $\delta^{13}C$ values were greatest in low rainfall regions, in the driest months of autumn, and in trees growing on shallow soils, or soils with major impediments to root growth.

Large variations in $\delta^{13}C$ in leaves and wood have been found within rainfall regions due to differences in soil water availability. For example, Högberg *et al.* (1993, 1995) found relatively large $\delta^{13}C$ (to -20.2 ‰) in foliage of *Pinus sylvestris* growing on soils with a hardpan or compacted layer of stones and gravel within 50 cm of the surface, whereas $\delta^{13}C$ in adjacent trees were up to 5 ‰ less. Available soil water also explained much of the seasonal and site variations in tree-ring $\delta^{13}C$ in *Pinus radiata* in New Zealand (Walcroft *et al.*, 1997).

Shallow soils and those with heavy texture that limited the amount of plant-available water storage, are typical of young soils formed directly on granite or gneiss along incised valleys in this region (Tille, 1996) and were a predisposing factor in drought death of *Pinus radiata* plantations (McGrath *et al.*, 1991). In April 1998, a large number of *E. globulus* died of drought in the Blackwood River valley on soils with less than 2 m depth. Growth of *E. globulus* is heavily dependent on the amount of stored soil water, especially where mean annual rainfall is less than 800 mm (Weston 1991; Hingston and Galbraith, 1998; Hingston *et al.* 1998; Shedley *et al.*, 1998). Hingston *et al.* (1998) found that four-year-old *E. globulus* used all the available soil water stored (210 mm) where soil depth was only 1.5 m over rock. Soils remained at wilting point for about 20 days during which time a large proportion of trees died. In contrast, where soils were deep, water was apparently extracted to at least 6m, with no evidence of growth limitation. It appears from this work and other studies (Hingston and Galbraith, 1998) that a minimum of 2 – 3 m of soil is required to store sufficient soil water (200 – 300 mm) for tree growth during the dry months each year.

The range of phloem $\delta^{13}C$ found in this study was greater than previously recorded. $\delta^{13}C$ for temperate C_3 plants lie typically within the range -26 ‰ to -30 ‰ (Francey *et al.*, 1985; Garten and Taylor, 1992; Stewart *et al.*, 1995; Newton *et al.*, 1996; Damesin *et al.*, 1997). Greater $\delta^{13}C$ values (-20 ‰ to -26 ‰) have been found in arid-zone plants (Ehleringer and Cooper, 1988; DeLucia and Schlesinger, 1991). In one study of *E. globulus*, foliage $\delta^{13}C$ was always less than -25 ‰ (Osório and Pereira, 1994) while in another, more recent study in the same region as the present, wood $\delta^{13}C$ ranged from -22 ‰ to -28 ‰ (Macfarlane and Adams, 1998). Phloem sap may show a greater range of $\delta^{13}C$ values than foliage and wood because photosynthates are still produced (and used to maintain basic metabolism) even when plants are chronically short of water, whereas production of new foliage or wood under such conditions is minimal.

Increased concentrations of sugars in phloem of *E. globulus* were almost invariably associated with poor availability of soil water and greater $\delta^{13}C$, regardless of nutrient status. Quick *et al.* (1992) have previously shown that rates of sugar production and export from leaves of *E. globulus* were reduced by shortages of water, even though the concentration of sucrose in leaves was increased. It seems likely that increased phloem sugar concentrations are the result of a change in the strength of the sink rather than the source. Concentrations of simple sugars in phloem probably increase as the demand in growing tissues for sugars declines (Fisher, 1983; Catesson *et al.*, 1995). Losses of water from the phloem to the transpirational stream (Downes *et al.*, 1999) may further contribute to the increased sugar concentration. Nevertheless, the strength of the sugar:$\delta^{13}C$ relationship was surprising and also suggests a synchronous relationship with increasing shortage of water between the reduction in growth and demand for sugars, and the reduction in stomatal conductance, possibly mediated by abscissic acid (Turner, 1986; Bunce, 1996).

Poor nutrition may limit sugar concentrations in phloem sap, owing to the indirect effects of reduced leaf area and photosynthetic capacity on sugar production, as well as direct reductions in sugar translocation from nutrient deficient leaves (Hartt, 1969; Doman and Geiger, 1979; Cakmak *et al.*, 1994). Eliminating plots of trees with clearly deficient concentrations of nutrients in foliage improved the relationship between tree growth and phloem sugar concentrations (see Chapter 4). This is because trees in which growth is limited by poor nutrition will tend to have lesser phloem sugar concentrations, whereas trees in which growth is limited by poor water availability will tend to have greater sugar concentrations, than trees with no growth limitations.

Interpretation of foliar $\delta^{13}C$ can be confounded by shading (e.g. Ehleringer *et al.*, 1986), re-fixation of soil-derived CO_2 which is strongly depleted in ^{13}C (e.g. Berry *et al.*, 1997), gradients in leaf-to-air vapour pressure deficits (e.g. Farquhar *et al.*, 1989) and to the effects of branch length on hydraulic conductivity and stomatal conductance (e.g. Walcroft *et al.*, 1996, Warren and Adams, 2000). Variations in foliar $\delta^{13}C$ values may also be attributed to differing environmental conditions when photosynthates are assimilated during leaf development (Meinzer *et al.*, 1992; Damesin *et al.*, 1997). Hence, a number of factors may have contributed to the 3 to 4 ‰ greater $\delta^{13}C$ of youngest leaf tips than mature leaves sampled from the same branch at the Klopper plantation. Most likely, the majority of the carbon in mature leaves was laid down during winter and spring, when water supply was non-limiting, whereas young leaf tips at branch ends and at the tree apex, which were produced during the dry months of summer and autumn, were composed of recently assimilated carbon. The 1 – 3 ‰ greater $\delta^{13}C$ values in the most recently assimilated carbon in phloem sap in the main stem than in leaf tips, and the 1 ‰ increase in phloem $\delta^{13}C$ with stem height, are fully consistent with the scenario of increasing water stress during autumn.

Phloem sap collected from the main stem has several advantages over foliage for determining current water availability based on $\delta^{13}C$, in that phloem sap is: (i) simpler to collect, store and prepare for analysis, (ii) representative of current photosynthate, (iii) composed of simple carbon compounds, and (iv) fluid and mobile - carbon loaded from source leaves of various ages and canopy positions is combined in the main stem. $\delta^{13}C$ of phloem collected from stems is thus representative of the whole canopy and has less within-tree variability than foliar $\delta^{13}C$.

A further advantage of using phloem to assess $\delta^{13}C$ and water availability is that the dominant simple sugars (sucrose and raffinose) are direct products of the initial carboxylation pathway via Rubisco (Farquhar *et al.*, 1982; Farquhar *et al.*, 1989) and their carbon content is subject to little further isotopic fractionation. Brugnoli *et al.* (1988) measured ^{13}C discrimination in soluble sugars in poplar leaves, produced in a single day, and found strong correlations between $\delta^{13}C$ and the ratio of intercellular to atmospheric CO_2 partial pressures (C_i / C_a), as predicted by Farquhar *et al.* (1989). Discrimination against the heavier isotope during other biochemical pathways can produce $\delta^{13}C$ signatures of whole leaf or wood tissues that are markedly different to those of recently fixed carbon (O'Leary, 1981; Leavitt and Long, 1982; Brugnoli *et al.*, 1988; Farquhar *et al.*, 1989).

Many of the soils within plots in the Klopper plantation had low C:N ratios (< 15:1), suggesting fast rates of nitrogen turnover and good availability of inorganic nitrogen (Richards, 1976; Adams and Attiwill, 1986). A few plots had concentrations of soil nitrogen < ~0.15 % and larger C:N ratios (16 - 23). As might be expected, concentrations of amino acids were less in trees growing on sandy soils with a low capacity to supply nitrogen (K7, 8, 15, and 16), and greatest in trees growing on high nitrogen soils (e.g. K2 and 17). However, very low concentrations of amino acids were also found in sap from trees with high $\delta^{13}C$ values, growing in soils with a high nitrogen supplying capacity (K3, 10, 11 and 12). Uptake of nitrogen from soils depends not only on availability of inorganic N, but also on soil moisture since this dictates the rate of movement of nitrogen to plant roots by both mass flow and diffusion (Nye and Tinker, 1977). These red loamy soils were also very dry and hard setting, which obviously limited the ability of fine roots to access available nitrogen. In most plantations in south-west Western Australia, the bulk of annual nitrogen uptake is met from the top 10 cm of soil (O'Connell and Rance, 1999), and so the structure and moisture content of the topsoil will thus play major roles in determining nitrogen availability and phloem concentrations of amino acids.

On the other hand, increased concentrations of amino acids in sap were found in trees growing on sandy soils (K13 and K14) which apparently had low capacity to supply nitrogen, and which were also depleted in soil moisture. These trees had relatively high foliar N concentrations (2.08 and 1.93 % N, respectively) due to recent heavy applications of fertiliser in this area by the previous landowner. It is possible that increased concentrations of amino acids in this case were maintained in phloem sap by remobilisation of soluble storage proteins in leaves (i.e. Rubisco), or in woody stems or roots (i.e. vegetative storage proteins). Other trees in this plantation had considerably less foliar N and may not have had the capacity to remobilise excess soluble nitrogen.

The relatively large concentrations of amino acids in trees with high foliar N status, but with poor water supply, could also result from lower demands for growth, resulting in an accumulation of both amino acids and sugars in phloem sap. This could have important implications for parrot damage, in that under these circumstances, phloem sap would be nutritionally enriched and highly attractive to parrots. Trees in the immediate vicinity of K13 and K14 had been badly damaged by parrots, even during their first year of growth. The concentrations of amino acids at K17 and N1 in autumn (20.2 and 19.1 μmol ml^{-1}) were also greater than expected for the level of water stress (-23.7 and -23.1 ‰). Trees in both these plots were growing on very fertile soil and were severely damaged by parrots in autumn, with an average strip length of greater than 50 cm per tree (see Chapter 4).

5.5 Conclusions

The concentrations of amino acids and sugars in phloem sap depend not only on the storage and accessibility of water and nitrogen in soils, but also on the production and translocation of photosynthates and amino compounds within trees, and on the growth requirements of trees. These varied considerably between sites and seasons, and between soil types, but the overriding environmental variable affecting phloem composition in this study was water stress. Severe water stress invariably increased sugar concentrations, and usually decreased amino acids concentrations, sometimes dramatically, and this had a significant effect on the C:N ratio. Greatest concentrations of amino acids were found where trees were well supplied with nitrogen, whether by heavy applications of fertiliser or from excessive accumulation of animal manure. In those situations, concentrations of amino acids remained high even when tree growth was limited by soil moisture, possibly due to an enhanced capacity to remobilise storage protein. It is in such situations that some of the most severe parrot damage has been recorded.

The use of $\delta^{13}C$ signatures in phloem sap is a relatively simple yet sensitive indicator of the current level of water stress in even-aged plantation grown *E. globulus*. It has the potential to separate out the effects of water stress and nutrient limitations on tree growth, and is currently being used for this purpose in current research work in Western Australia. Phloem sap analysis may be a useful technique for rapid assessment of the nutrient status of *E. globulus* and provide information on nutrient movement within the translocatory system. It should also assist in the understanding of tree selectivity by various phloem sap-feeders.

CHAPTER 6

EFFECTS OF NITROGEN, POTASSIUM AND PHOSPHORUS FERTILISERS ON PHLOEM COMPOSITION IN *EUCALYPTUS GLOBULUS*

6.1 Introduction

One of the reasons that plantations of *E. globulus* have been grown so successfully around the world on vastly differing soil conditions is the ability of this species to respond to fertiliser (Cromer *et al.*, 1981; Schönau and Herbert, 1989; Judd *et al.*, 1996). In Western Australia, plantations of *E. globulus* are routinely fertilised at establishment, even though many are planted on ex-farmland where the availability of N and P in the soil has been increased by years of fertiliser additions (especially P) and growth of leguminous pastures. Despite a continuing cataloguing of 'fertiliser responses', the physiological mechanisms responsible for the increase in growth have not been thoroughly examined. Long-distance transport is a key element of the nutritional physiology of all plants and only recently has there been concerted effort to characterise xylem and phloem transport of nutrients in eucalypts (Pate and Arthur, 1998; Pate *et al*, 1998; Pate and Arthur, 2000; see also Chapters 4 and 5).

Applications of nitrogen in excess of growth requirements are known to increase the accumulation of amino acids, especially arginine (Tromp and Ovaa, 1979; Näsholm *et al.*, 1994; Adams *et al.*, 1995), in stems and foliage. This phenomenon, as well as a putative increase in nitrogen concentrations in phloem sap, has long been linked with outbreaks of sap-feeding and leaf-eating insects in eucalypts (e.g. Landsberg *et al.*, 1990; Adams and Atkinson, 1991). Similar hypotheses have linked the feeding patterns of arboreal marsupials and nitrogen availability in eucalypt foliage (Braithwaite *et al*, 1983; Landsberg, 1987; Cork, 1992). More generally, applications of fertilisers, particularly nitrogenous fertilisers, may increase the susceptibility of trees to browsing damage by a variety of animals (Gessel and Orians, 1966; Andersson *et al.*, 1970; Brockley, 1988; Gill, 1992; Bryant and Raffa, 1995).

The effect of addition of fertiliser of known composition on phloem sap composition in eucalypts has yet to be evaluated. Recently, Pate and Arthur (1998) reported the effects of irrigation with treated urban effluent on phloem sap of *E. globulus*. Although the effects of nutrients were confounded by the application of water, the nitrogen provided in the effluent led to high concentrations of amino acids in phloem sap throughout the year. In a parallel analysis in a rain-fed plantation, concentrations of amino acids in phloem sap plummeted shortly after rainfall ceased, and topsoils dried out, in early summer (Pate and Arthur, 1998). In a previous study (Chapters 4 and 5), the potential utility of phloem sampling and analysis for diagnosis of water and nutrient availability was illustrated. This study examines the effects of fertiliser additions on phloem sap composition of *E. globulus* in two trials based on sites differing in availability of soil water and nutrients. Additional aims were to examine the effects, if any, of fertiliser additions on carbon isotope ratios ($\delta^{13}C$) in phloem sap, and on tree growth and damage to trees by *Barnardius zonarius* parrots.

6.2 Methods

6.2.1 Site descriptions

Study sites were selected in August 1996 within the Klopper plantation managed by Bunnings Treefarms. Two trials were conducted, one using trees planted in 1994 and the other using trees planted in 1995. The 1994 trial included previously described plots K2, K7 and K15 that varied in soil water and nutrient availabilities (see Chapters 4 and 5 for full description of site characteristics). Briefly, K2 has deep, well structured red loam soil with high moisture storage capacity and high nutrient status, whereas K15 has deep sandy duplex soil with moderately high soil moisture capacity and low nutrient status. The poorest site (K7) has moderately deep sandy gravel duplex soil overlying partially cemented ironstone. This soil type has low soil moisture storage capacity, difficult rooting conditions and low soil nutrient status. The 1995 trial was located in a compartment adjacent to that containing K15, but had site characteristics similar to those at K7, with sandy gravel duplex soils of varying depth, hardness and nutrient status.

6.2.2 Experimental design

The 1994 trial consisted of five addition treatments (N, P, K, NPK, Control), replicated three times (15 plots), in a single randomised block at each of the previously described sites K2, K7 and K15. Each plot consisted of nine adjacent trees at a spacing of 1.5 m. Plots were separated by at least two unfertilised 'buffer' trees or buffer rows. Treatments were randomly assigned within the experimental site. Fertilisers were applied in September 1996 using urea (46 % nitrogen), muriate of potash (49.5 % potassium, 50 % chloride) and All Phos® (20 % phosphorus, 15 % calcium). Each fertiliser was applied on the appropriate plots, at the following rates: 250 kg N ha^{-1}, 108 kg P ha^{-1} and 200 kg K ha^{-1}. The NPK plots received all three fertilisers at the above rates, while control plots were left unfertilised. Fertilisers were weighed in the field and spread by hand evenly along tree rows, to approximately 100 cm either side of trees.

The 1995 trial consisted of six paired plots (P1 – P6) located approximately equidistant (30 m) from the compartment edge, and surrounding a remnant of native vegetation, in which parrots were numerous. Each pair consisted of two adjoining plots of 50 trees (five rows x ten trees), separated by two rows of unfertilised 'buffer' trees. One plot in each pair was treated with fertiliser (i.e. P1+, P2+, etc), while the other, control plot, remained unfertilised (i.e P1-, P2-, etc). Surface soils (to 10 cm depth, sampled in each plot prior to fertiliser application) contained moderately high concentrations of total nitrogen (0.31 % ± 0.04) and available phosphorus (49 $\mu g\ g^{-1} \pm 6$), and adequate concentrations of available potassium (93 $\mu g\ g^{-1} \pm 9$). There were no significant differences between treatment and control plots for any of the soil parameters analysed. Rates of N, P and K were the same as described for the 1994 trial, although N and P were applied together as Agras® (17.5 %N, 7.6 % P, 17.0 % S), rather than as separate applications of urea and All Phos®. Herbicide was sprayed in the inter-row strips in August 1996 to control pasture growth.

6.2.3 Tree growth and parrot damage measurements

All trees in the 1994 trial were measured for diameter at breast height (DBH) using a diameter tape, and total height (HT) using a telescopic height stick, in October and December 1996, and in February, May, and November 1997. In the 1995 trial, twenty internal trees per plot were measured for DBH and HT in November 1996 and in January, March, May and September 1997. Stem volumes (V) were estimated using the conic function $V = 1/3\ (BA*HT)$, where BA is the basal area at breast height. Parrot damage to trees was recorded at each measurement time for the 1995 trial by counting the number of trees recently damaged per plot, and by estimating the cumulative length of recent stripping on the central leader or replacement leader, as described in Chapter 2. The maximum height at which damage occurred on the stem was recorded at each assessment, so that only new or recent damage above this height was included in subsequent assessments.

6.2.4 Phloem sampling and analyses

Phloem sap was collected from incisions cut in the main stem of trees 1 - 2 m above ground, as described in Chapter 4 (Section 4.2.2). Approximately 20 - 30 μl of sap was collected in microcapillary tubes Drummond Microcaps) from each tree and combined in 1.5 ml Eppendorf vials to give one bulk sample per plot. A subsample was taken for the determination of total sugar concentration using an Erma temperature-compensated hand refractometer (0 - 32% sucrose equivalents w/v). Samples were then kept on ice in the field and later stored at -20° C until required for analysis. Phloem sap was sampled in the 1994 trial in October and November 1996 and in February and April 1997. In the 1995 trial, sap was sampled from each plot in October and November 1996 and in January and March 1997.

All phloem sap samples were analysed for total amino acid concentration, using the ninhydrin colorimetric method described by Yem and Folkes (1958). This method is sensitive, and relatively inexpensive. Briefly, 3 μl of phloem sap was mixed with 497 μl of de-ionised water in 5 ml borosilicate glass tubes to which was then added 1 ml of 0.2 M citrate buffer (pH 5.0) plus 1 ml of the ninhydrin reagent (see Yem and Folkes (1958) for details). Tubes were then incubated (sealed with a marble) in a boiling water bath for 12 minutes, and for a further 10 minutes at room temperature, before reading absorbance using a spectrophotometer set at 570 nm. Each sample was analysed in duplicate, and each sample batch included blanks and a range of standard solutions of asparagine and glutamine.

Potassium concentrations in phloem sap were determined by atomic absorption spectrometry (AAS), as described in Chapter 4 (Section 4.2.3). Phosphorous concentrations were determined using a colorimetric assay following the method of Murphy and Riley (1962). For this assay, 50 μl of sap was diluted with 1.95 ml DDI, then 2 ml of mixed reagent (ammonium molybdate + potassium antimony tartrate) and 1 ml ascorbic acid reagent (1.76 g made up to 100 ml in DDI) was added and mixed well. Absorbance was read at 650 nm after colour development for 30 min.

Phloem $\delta^{13}C$ values were determined by mass spectrometry as described in Chapter 5 (Section 5.2.5). All samples in the 1995 trial, and all plots at K7 in the 1994 trial sampled in February, and a representative sample from each treatment collected in October, November and April, were analysed for $\delta^{13}C$.

Amino acid composition was determined by HPLC (see Chapter 4 for details) for each sampling time in the 1994 trial using one replicate sample per treatment.

6.2.5 Foliage sampling and analysis

Leaves were sampled from the upper crown by cutting at least six branches per plot, and collecting 40 youngest fully expanded leaves (YFELs) per sample. Trees at K15 were sampled in January 1997, while trees at K7 were sampled in February 1997. All leaves were placed in paper bags and oven-dried at 70° C for three days, then sent to the CSBP Laboratories in Perth for elemental analysis.

6.3 Results

6.3.1 Growth responses to fertiliser application

In the 1994 trial, fertiliser had no significant effect on tree growth during the time frame of the study (Table 6.1). Growth was greatest at K2 (Fig. 6.1), the site known to have the greatest concentrations of total N in the soil (0.56 % cf 0.14 % for K7 and 0.16 % for K15). By the time of final measurement, tree volumes differed significantly among sites (F = 62.8, $P<0.001$). Similarly, there were no growth responses to fertiliser in the 1995 trial (Table 6.2).

6.3.2 Foliar responses to fertiliser

Potassium concentrations in YFELs sampled in January at K15 were significantly greater ($P<0.01$) in trees receiving K (0.80 %) and NPK (0.78 %) than in unfertilised trees (0.54 %) (Table 6.3). This response was expected given the critically low foliar K concentrations previously found at this site (i.e. 0.42 %, March 1996). N and P fertilisers also tended to increase foliar concentrations of the nutrients applied, but these were not significantly greater than in unfertilised trees. Application of NPK fertiliser at K7 increased foliar concentrations of N, K and S ($P<0.01$) above those in unfertilised trees, while addition of P alone increased the foliar concentration of K ($P<0.01$) (Table 6.3).

Table 6.1
Summary of tree volumes at three sites in the 1994 trial measured in November 1997, 14 months after application of N, P and K fertilisers. Treatments were N, P, K, NPK and C (control, i.e. no fertiliser), replicated three times. Each value is the mean volume (m^3 10^{-3}) of nine trees per plot. There were no significant differences in growth among treatments.

Site	Rep	N	P	K	NPK	C
K2	1	61.61	66.98	70.32	60.50	52.72
	2	57.15	63.60	63.13	51.48	61.22
	3	66.26	61.40	51.86	46.65	76.22
	Mean	61.67	63.99	61.77	52.88	63.39
	SE	2.63	1.62	5.37	4.06	6.87
K7	1	44.05	35.86	32.72	38.07	35.49
	2	44.94	27.55	36.94	35.55	44.17
	3	40.80	28.81	28.49	32.35	40.43
	Mean	43.26	30.74	32.72	35.32	40.03
	SE	1.26	2.59	2.44	1.66	2.51
K15	1	41.12	42.60	37.26	52.47	41.34
	2	42.78	45.44	31.47	42.65	51.84
	3	50.31	48.95	47.43	47.60	49.12
	Mean	44.74	45.66	38.72	47.57	47.43
	SE	2.83	1.84	4.66	2.83	3.1

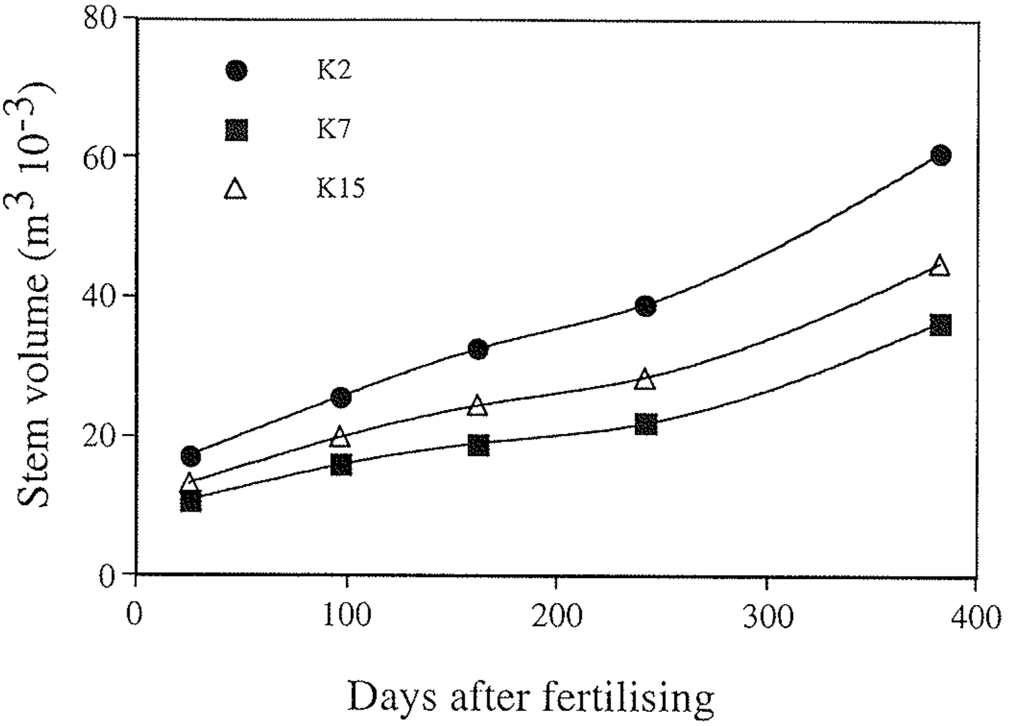


Figure 6.1 Volume growth curves for trees at three sites at the Klopper plantation following fertiliser application in September 1996 (Day 0). Each data point is the mean volume of 135 trees per site.

Table 6.2
Summary of tree volumes in the 1995 paired plots trial measured in January and May 1997, following application of NPK fertiliser in September 1996. Plots receiving fertiliser are designated 'NPK' while those receiving no fertiliser are designated 'Control'. Each value is the mean stem volume ($m^3\ 10^{-3}$) of 20 trees per plot. There were no significant differences in growth between treatments.

Plot	January		May	
	NPK	Control	NPK	Control
1	3.69	3.18	8.72	8.14
2	2.81	2.78	6.50	8.00
3	3.02	3.38	8.68	9.36
4	3.98	4.25	10.93	10.13
5	4.45	4.53	10.88	10.75
6	4.52	4.60	9.70	10.53
Mean	3.75	3.79	9.23	9.48
SE	0.29	0.31	0.68	0.49

Table 6.3
Foliar concentrations of N, P and K in YFELs collected from the 1994 trial at (a) K15 in January 1997, and (b) K7 in February 1997. Values represent the means of three replicate plots (± SE). Foliar concentrations that differ significantly from those in unfertilised trees within each site (****P*<0.001, ***P*<0.01, **P*<0.05).

Site	Treatment	N%		P%		K%	
		Mean	SE	Mean	SE	Mean	SE
K15	N	2.23	0.13	0.16	0.01	0.64*	0.03
	P	2.03	0.07	0.16	0.00	0.74	0.09
	K	1.74	0.02	0.13	0.00	0.80**	0.04
	NPK	2.32	0.07	0.17	0.01	0.78**	0.04
	C	1.90	0.14	0.15	0.01	0.54	0.01
	Mean	2.04		0.15		0.70	
K7	N	1.89	0.17	0.13	0.01	0.92	0.07
	P	1.68	0.05	0.13	0.00	1.07**	0.03
	K	1.74	0.08	0.11	0.00	1.01	0.07
	NPK	2.01**	0.03	0.13	0.00	1.03**	0.03
	C	1.55	0.09	0.11	0.00	0.85	0.01
	Mean	1.78		0.12		0.97	

6.3.3 Phloem sap δ^{13}C values

Analysis of phloem sap δ^{13}C values showed that growth at the Klopper plantation was strongly affected by tree water stress caused by poor soil water availability (see also the previous chapter). In the 1994 trial, δ^{13}C values increased from -26.8 ‰ in October to an unusually high (less negative) value of -20.9 ‰ in April at the most severely stressed site (K7) (Fig. 6.2). This seasonal change was paralleled by an increase in sugar concentration of almost 6 % (w/v). Similarly, in the 1995 trial, mean phloem δ^{13}C values increased from -25.9 ‰ in November to -21.6 ‰ in January, but then decreased to -23.4 ‰ in March following some unseasonal rain. Tree volume measured in January was strongly related to current phloem sap δ^{13}C values ($r^2 = 0.504$, $P<0.01$) in the 1995 trial. There were no significant differences in mean δ^{13}C values between fertilised and unfertilised plots in either trial at any sampling time. Foliar concentrations of nitrogen in youngest fully expanded leaves (YFELs), sampled from the upper crown in February at K7, were significantly greater ($P<0.01$) in the NPK plots (2.01 % N) than in unfertilised plots (1.55 % N) (Table 6.3), but this was unrelated to phloem δ^{13}C (data not presented).

6.3.4 Amino acid concentration and composition

In the 1994 trial, addition of N and NPK fertilisers increased the concentration of amino acids in phloem more than two-fold at K7 and K15, but had no effect at the more fertile site, K2. However, concentrations of amino acids in all plots declined dramatically to a minimum of 10 μmol ml^{-1} by February as soils dried out. Addition of N still had an effect on concentrations of amino acids in April at K15 (Fig. 6.3), six months after fertiliser application.

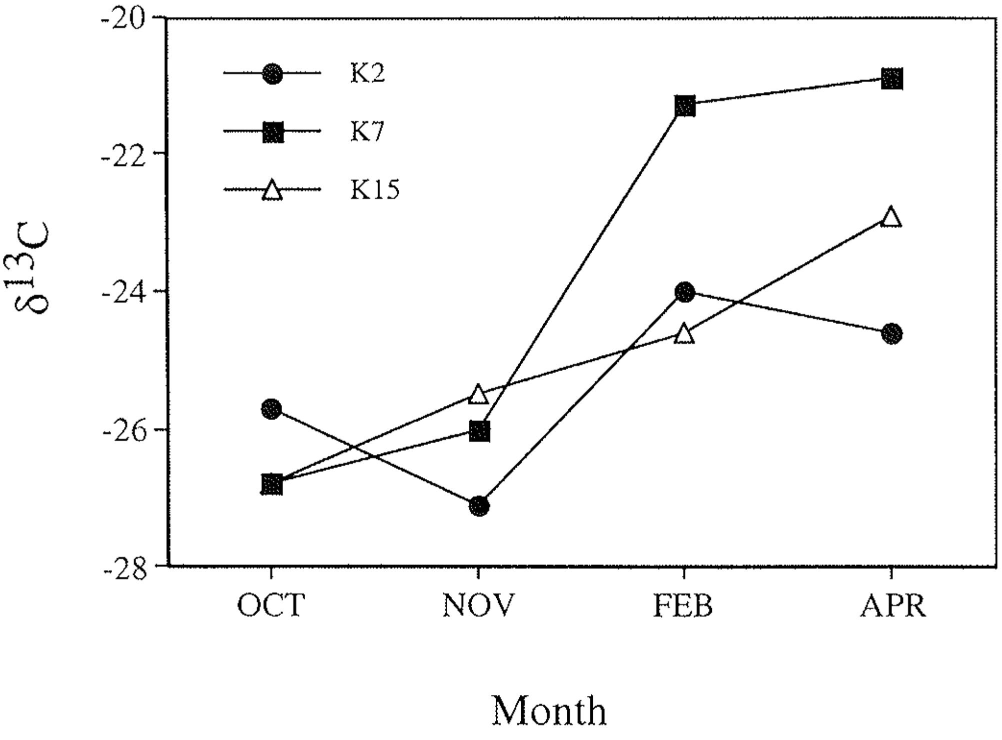


Figure 6.2 Seasonal variation in carbon isotope composition ($\delta^{13}C$) in phloem at three sites in the 1994 Klopper plantation. Each data point is the mean value of four plots, i.e. one complete replicate block per site.

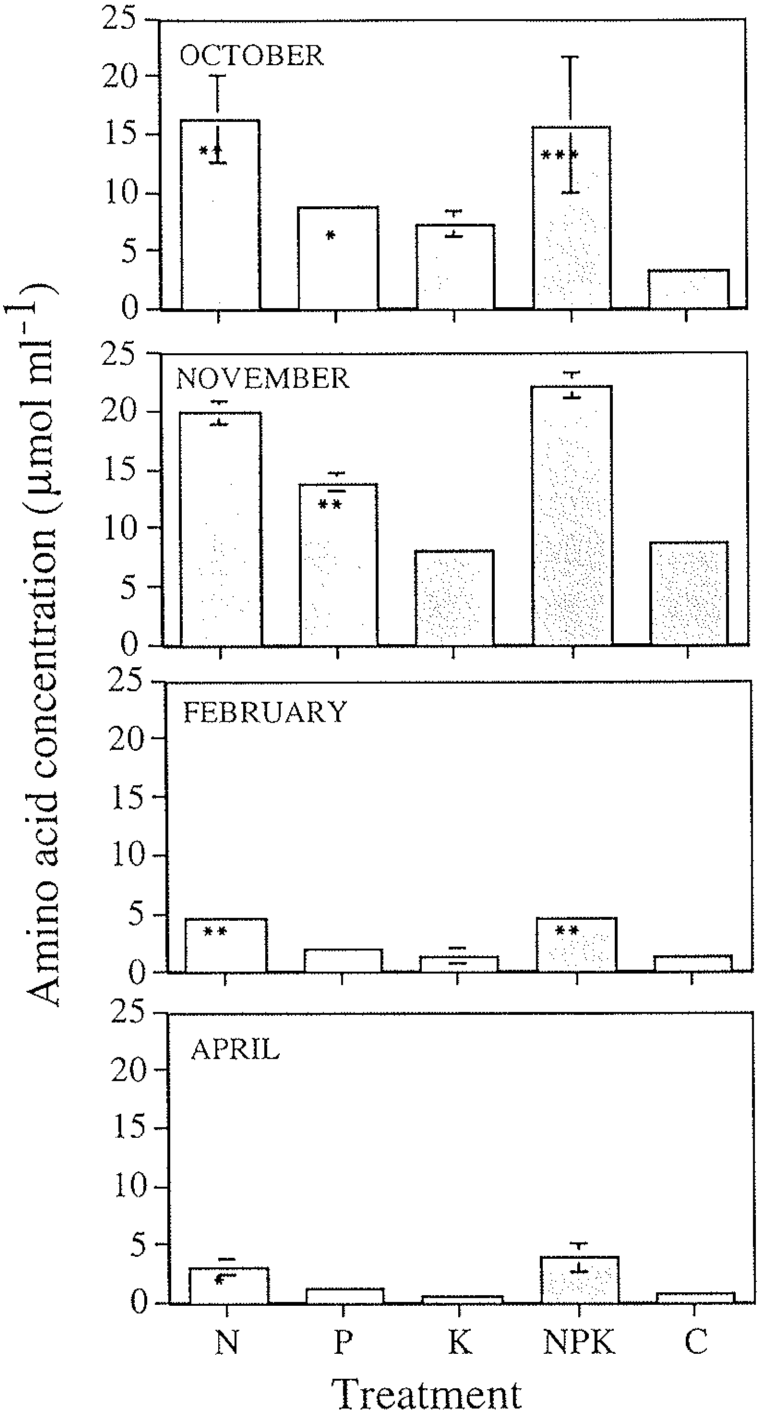


Figure 6.3 Concentration of amino acids in phloem sap following application of fertiliser (N, P, K and NPK) at K15 in the 1994 Klopper plantation. Each bar is the mean value from three replicate plots, with standard error bars. Amino acid concentrations in fertilised plots that were significantly different from those in unfertilised plots (C) are shown: $***P<0.001$, $**P<0.01$, $*P<0.05$.

In the 1995 trial, addition of NPK fertiliser significantly increased the mean concentration of amino acids in October ($P<0.001$), November ($P<0.05$) and March ($P<0.05$), but not in January (Fig 6.4). Concentrations of amino acids were exceptionally large in fertilised trees in October (28 - 44 μmol ml^{-1}), within one month of fertiliser application. The effect of fertiliser on amino acid concentrations was relatively short-lived at P5 and P6, whereas at P2, fertilised trees maintained greater concentrations of amino acids until January, and at P1, this effect was still evident in March.

On 19th November 1996, bulk samples of phloem sap were collected near P3 (in the 1995 trial) from sixteen even-sized unfertilised trees (height approximately 3 m) at 0 - 1, 1 - 2, and 2 - 3 m stem heights, and were found to have concentrations of amino acids at these respective stem heights of 15.9, 15.4 and 25.5 μmol ml^{-1}. Based on this proportional increase, concentrations in the upper stem position at P1+ in October might have been as high as 70 - 80 μmol ml^{-1}.

Amino acid composition at K7 in the 1994 trial varied with sampling time and type of fertiliser. Glutamine was the dominant amino acid in spring when soil nitrogen availability could be expected to be greatest. This amide comprised up to 80 % of total amino N in the NPK plots, but this proportion decreased to less than 20 % in February, when the concentration of total amino acids was only 2.5 μmol ml^{-1}. At the latter time, concentrations of aspartic acid and glutamic acid were greater than that of glutamine. Other qualitative variations in amino acid composition were observed at K7. In particular, the concentration of arginine in November was 7 and 10 times greater in the N and NPK plots, respectively, than in the unfertilised plot, but was absent from all samples by February.

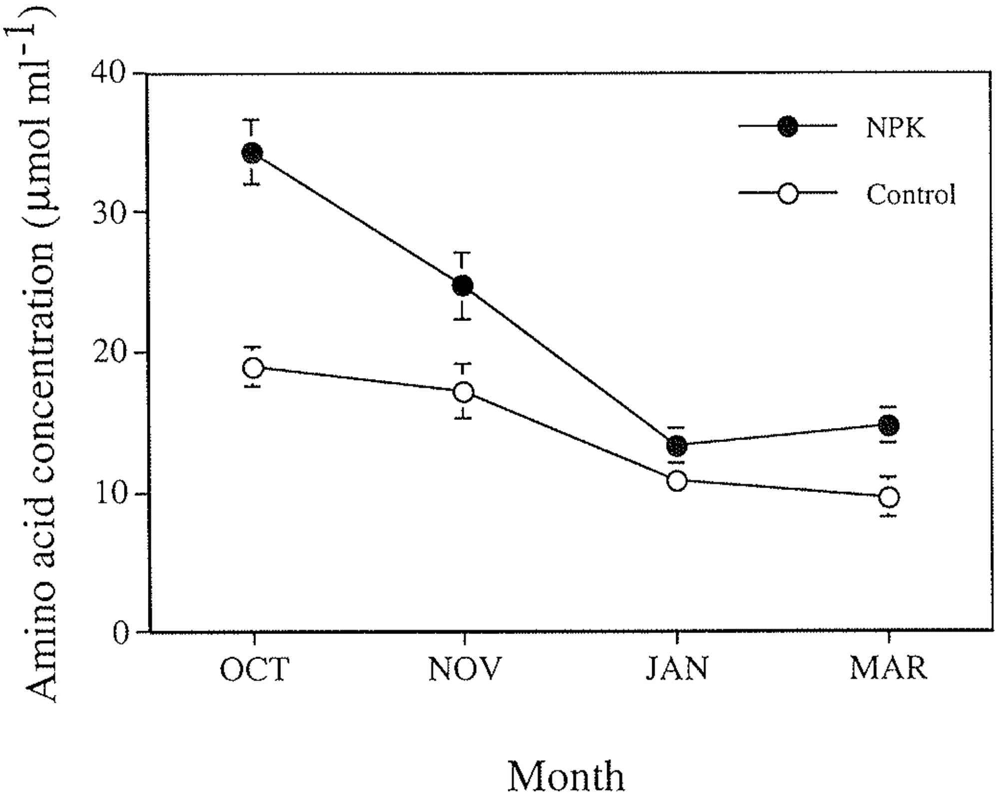


Figure 6.4 Mean concentrations of total amino acids in phloem sap collected from *E. globulus* at the 1995 Klopper plantation site, between October 1996 and March 1997. Each data point represents the mean of six plots, one bulk sample collected per plot of 20 trees.

6.3.5 Effects of fertiliser on concentrations of potassium and phosphorus in phloem sap

Concentrations of potassium in phloem sap generally differed significantly among sites and seasons in the 1994 trial, but not among fertiliser treatments. Only in April at K15 were concentrations of K in phloem sap greater in fertilised plots than in unfertilised plots (Fig 6.5). There was no significant effect of fertiliser on potassium concentrations in the 1995 trial.

In the 1994 trial, there was a short-lived increase in phloem sap P in response to fertiliser. Addition of NPK fertiliser increased the concentration of phosphate above that in unfertilised plots at each site in October, as did addition of P at K15, and N at K7. However, by November there were no differences among treatments (data not presented).

6.3.6 Effect of fertiliser on total sugar concentrations in phloem sap

In the 1994 trial, sugar concentrations varied significantly among sites at each sampling time (October and April, $P<0.01$; November and February, $P<0.001$), and between sampling times ($P<0.001$). Greatest seasonal differences were at K7, where mean sugar concentration increased from 20.9 % in October to 26.7 % in April. However, addition of fertiliser had no effect on sugar concentrations at K7 or K15, and only a slight effect at K2 in October, where trees fertilised with N or P ($P<0.05$), or NPK ($P<0.01$), showed lesser concentrations of sugars than in unfertilised trees (Fig. 6.6). This effect was not evident at any other time. There was no significant effect of NPK addition on sugar concentrations in the 1995 trial.

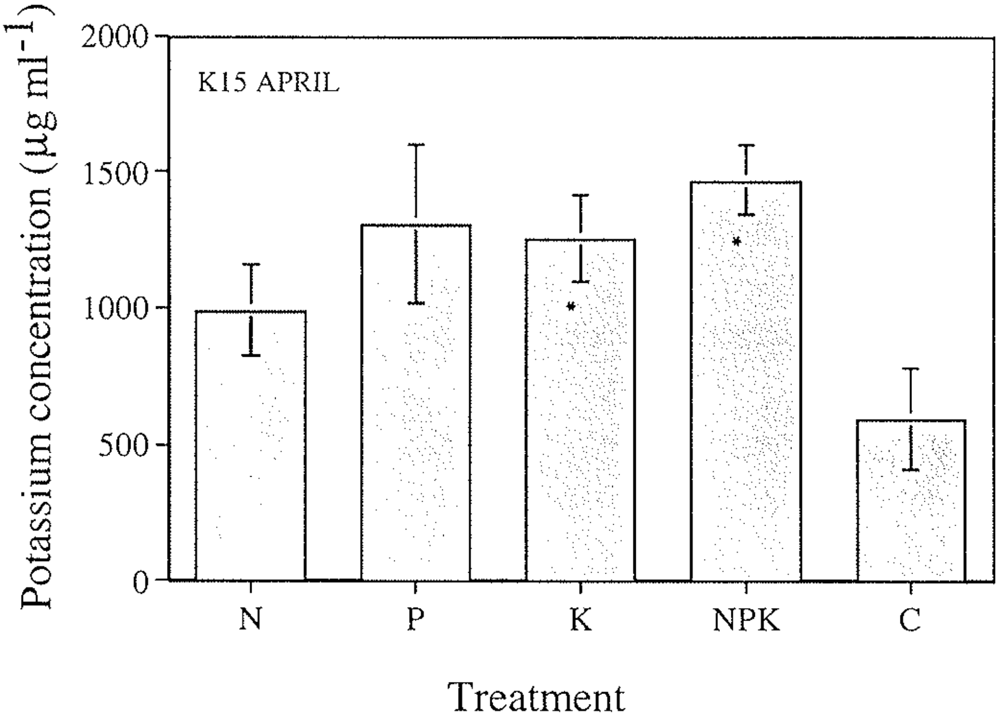


Figure 6.5 Effect of fertiliser on the concentration of potassium in phloem sap in April at K15 in the 1994 Klopper plantation. Standard error bars are shown. Concentrations of potassium in fertilised plots that were significantly different to those in unfertilised control plots (C) are shown: $*P<0.05$.

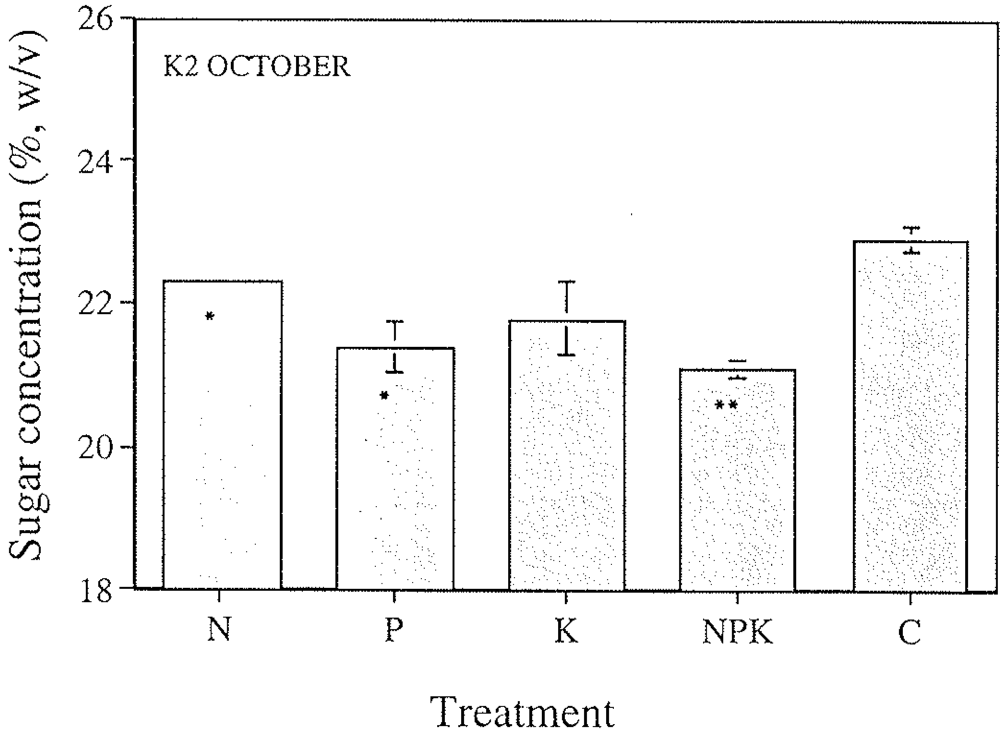


Figure 6.6 Effect of fertilisers on sugar concentrations in October 1996 at K2 in the 1994 Klopper plantation. Each bar is the mean concentration from three replicate plots, with standard error bars. Concentrations in fertilised plots that were significantly different from those in unfertilised plots (C) are shown: $^{**}P<0.01$, $^{*}P<0.05$.

6.3.7 Effect of fertiliser on parrot damage

Parrot damage was greatest during the February-March period, with a total of 2,350 cm bark stripped from 34 % of trees measured, giving a mean strip length in recently damaged trees of almost 30 cm per tree. Least damage was found during the September-November period with only 200 cm bark stripped from 9 % of trees measured. and a mean strip length of approximately 10 cm per damaged tree. However, this seasonal pattern of damage was not consistent for all plots. Trees in P2+ were severely damaged during the December to January period, whereas at four other plots (P4+, P4-, P5+, and P6+), no damage was apparent.

Damage was greater in fertilised trees at P2+ than in P2- for the December to January period ($P<0.001$), and tended to be greater in P1+ than P1- during the February to March period ($P = 0.072$), but overall differences in damage for all plots between fertilised and unfertilised trees were not significant at any time due to the large variability in intensity of damage between replicate plots (Fig. 6.7).

In May, cumulative damage per plot proved to be negatively related to mean tree volume ($r^2 = 0.698$, $P<0.001$, $n = 12$) in other words, damage was greater in plots with smaller trees (see Tables 6.2 and 6.4). This negative relationship may have been due to the effect of severe damage on height growth in some plots. Many trees in P2+ had broken tops as a result of parrot damage, which affected total tree height and hence stem volume estimates. However, within many plots, cumulative damage in May was significantly and positively related to stem volume (Table 6.5).

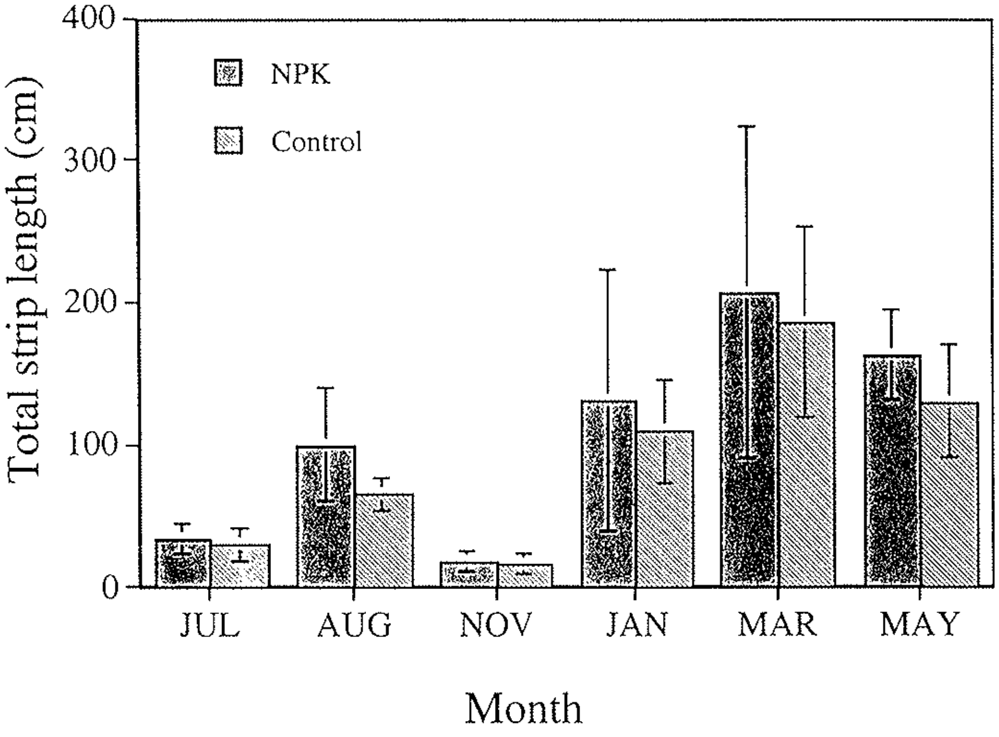


Figure 6.7 Recent parrot damage recorded in fertilised (NPK) and unfertilised (Control) trees for the periods shown between July 1996 and May 1997. Each bar is the mean damage of six replicate plots, with standard error bars.

Table 6.4
Cumulative parrot damage in fertilised (NPK) and unfertilised (Control) trees in the 1995 trial in January and May 1997. Each value is the total cumulative strip length (cm) of 20 trees per plot.

Plot	January		May	
	NPK	Control	NPK	Control
1	145	190	990	740
2	960	170	1515	675
3	340	305	660	670
4	55	110	325	185
5	150	425	185	680
6	50	120	240	265
Mean	283	220	652	536
SE	142	50	212	99

Table 6.5
Regression statistics for the relationship between cumulative damage per tree and tree volume, for each plot (n = 20 trees) measured in May 1997. Regression equations which were significant are shown: **P*<0.05, ***P*<0.01, ****P*<0.001, ns – not significant.

Plot	r^2	*P*	Plot	r^2	*P*
P1+	0.266	*	P1-	0.281	*
P2+	-	ns	P2-	-	ns
P3+	0.309	**	P3-	-	ns
P4+	-	ns	P4-	0.390	**
P5+	0.212	*	P5-	-	ns
P6+	0.545	***	P6-	0.282	*

6.4 Discussion

This study demonstrated that application of nitrogen fertiliser induces rapid, although transient increases in concentrations of amino acids in phloem sap of *E. globulus*. This response was encountered even where there had been no apparent response in tree growth over the twelve-month study period. Concentrations of amino acids increased two-fold following nitrogen application in spring, but then decreased markedly during late summer and autumn in all plots as soils dried out, and rates of nitrogen mineralisation and uptake from soils typically decrease. Addition of fertiliser had no significant effect on phloem sap $\delta^{13}C$ values and only a minor influence on concentrations of phloem sugars. This rapid decline in amino acid concentrations in phloem sap during summer is fully consistent with that reported by Pate and Arthur (1998) for a rain-fed plantation at Mt Barker, Western Australia.

The lack of growth response to addition of fertiliser in this study can be attributed to overriding effects of seasonal water stress at the Klopper plantation, as discussed in more detail in the previous chapter. Similarly, Weston (1991) found no growth responses to NPK fertiliser over a two-year period at two sites in Gippsland, Victoria, where rainfall was less than 800 mm. At the Klopper plantation, total rainfall during 1997 was less than 600 mm, and water stress was evident during autumn, particularly at K7, where $\delta^{13}C$ values increased to -20.9 ‰. This contrasts with other fertiliser trials with eucalypts (Cromer *et al.*, 1981; Cromer and Williams, 1982; Schönau and Herbert, 1982; Yost *et al.*, 1987; Birk and Turner, 1992; Judd *et al.*, 1996), conducted in high rainfall regions, where growth was primarily limited by nutrient availability.

Addition of nitrogen lead to sharp increases in amino acid concentrations in phloem sap. Addition of phosphorus also increased amino acid concentrations, but to a lesser extent, so the response to nitrogen plus phosphorus tended to be greater than for nitrogen alone. Potassium addition had no effect on amino acid concentrations, but temporarily decreased sugar concentrations at one site, possibly due to increased flow rates of phloem sap (Marschner, 1995). Increases in phloem nitrogen concentrations with increasing nitrogen supply have been found in non-nodulated *Lupinus albus* (Atkins *et al.*, 1979), and in *Beta vulgaris* (in Lohaus *et al.*, 1994). Similarly, the total amino N content of phloem exudates was increased some 50 % by exposure of beech seedlings to an ammonia-enriched amosphere (Gessler *et al.*, 1998). While there are no comparable studies of effects of fertiliser on phloem sap composition in eucalypts, the results strongly suggest that where tree growth is limited by availability of water, fertiliser applications are unlikely to produce sustained responses in amino-N content of phloem sap.

Although nitrogen fertiliser is shown in this study to increase concentrations of phloem sap amino acids during spring, the response was short-lived. However, small responses in terms of amino acid concentration in phloem sap were still evident at one site until April in the 1994 trial, and in one plot until March in the 1995 trial. This indicated that either the additional supply of nitrogen at these sites had simply been inadequate to sustain longer term responses, or that increasing levels of water stress at these sites in autumn had limited the uptake of additional nitrogen.

Previous research has suggested that $\delta^{13}C$ values of foliage may increase in response to nitrogen application if the foliar nitrogen concentration and photosynthetic capacity of leaves increases (Field and Mooney, 1986; Evans, 1989; Farquhar *et al.*, 1989). Alternatively, increased nitrogen availability may result in increased leaf area and greater demand for water, causing greater stomatal closure (and increased $\delta^{13}C$) if soil water availability is limited (Högberg *et al.*, 1993). The lack of an effect of fertiliser on phloem sap $\delta^{13}C$ in the present study, and the absence of a growth response (including leaf mass and area), argues against a secondary effect of nitrogen via increased transpiration. Results also support the view expressed in the previous chapter that the nitrogen status of leaves has only a minor influence on phloem $\delta^{13}C$ values, compared with the more direct effect of soil water availability.

Fertiliser applications also had no significant effect on tree damage caused by parrots. Adams (1996), in commenting on earlier suggestions by Cork *et al.*

(1990) that the nutritional quality of eucalypts for herbivores could be modified by manipulation of soil nutrients, suggested this was "too optimistic unless all other constraints to growth can be removed". The present results clearly support this view. Tree damage by parrots is also strongly seasonal and greatest from late summer to early winter, with little damage in spring (Ritson, 1995). Thus large differences in amino acids between fertilised and unfertilised plots during spring would have largely gone unnoticed as parrots were foraging for seed in nearby crops and pastures. Damage did not increase until after the cereal crop was harvested in late December, but by this time, concentrations of amino acids in phloem sap had decreased markedly, and there was little variation among treatments.

One might also suggest that the lack of growth response to nitrogen also limits the visual cues available to assist parrots in the recognition of trees with greater amino acid concentrations. Selection by parrots of the more nutritious stems may simply be a matter of recognising differences in tree height, leaf colour or canopy density in even-aged plantations. In other studies (Andersson *et al.*, 1970), increased rates of bird damage in trees receiving annual dressings of nitrogenous fertiliser over ten years may well have been associated with visual cues afforded by the significant increase in growth of fertilised trees. However, I have often observed parrots 'taste-testing' trees by just loosening the bark on apparently suitable stems. Those stems that do not appeal are left hardly touched, while parrots may spend anything up to 30 minutes feeding on others. Clearly, visual cues are not the only way that parrots select stems. For example, one might expect a feedback effect on choice once palatability is found particularly good, reinforcing the visual selection of trees based on the above criteria.

6.5 Conclusions

The lack of growth response to fertiliser by *E. globulus* is not surprising in this region of southwest Western Australia where the strongly Mediterranean climate, and high annual evaporation, combined with often limited soil depth, dictate that water availability is the major constraint to tree growth. While tree nutrient status can be manipulated to some extent by addition of fertiliser, there is no guarantee that parrots will detect the change without adequate visual cues. These problems are exacerbated in cases such as this where birds have a wide choice of food resources.

CHAPTER 7

SYNOPSIS AND FUTURE RESEARCH

7.1 Synopsis

There has been much conjecture in Western Australia as to why Twenty-eight parrots (*Barnardius zonarius*) damage stems of eucalypts, and why some plantation trees are seemingly highly attractive to parrots while others are left untouched. The data presented in this thesis strongly suggest that parrots are seeking a food supplement from the phloem and cambium in upper stems of eucalypts. This study has examined patterns of damage in even-aged plantations of *E. globulus*, as well as seasonal and spatial variations in phloem sap composition, and has identified a number of factors that may predispose plantation trees to parrot damage.

Clearly, of greatest significance is the strong relationship between parrot abundance and tree damage (Chapter 2). This finding suggests that control of parrot populations might appreciably reduce the severity of the 'parrot damage' problem. Since my research began, shooting programs on the twenty-eight parrot (an unprotected species) in the medium rainfall region appear to have been quite successful in reducing tree damage, particularly where it had been possible to reduce parrot numbers in the year before planting. However, a trial conducted over two years in the low rainfall region, where over 60,000 parrots have been shot and trapped from an area of 160,000 ha, has shown little reduction in damage to trees, and no significant reduction in parrot populations (Morgan and McNee, 1999). Obviously, more research is required to understand population dynamics of twenty-eight parrots and determine the nature of the density-dependent relationships between parrot abundance and damage in different areas.

'Distance to edge' satisfactorily explained observed differences in the level of damage in certain plantations. This parameter, together with 'distance from mature native trees', suggests that parrots tend to conserve energy when feeding by minimising flight distances. This strategy should be most beneficial during the breeding season, and during the hot, dry summer months when parrots may be forced to restrict their movements to avoid loss of body water during high daytime temperatures and periods of intense solar radiation. Where parrots are evenly distributed around a plantation, my study has shown that damage generally decreases with increasing distance from a plantation edge. However, in areas of high parrot abundance, trees may be damaged up to 300 m in from an edge.

Such knowledge might be used to improve the design of plantations so as to minimise risk of parrot damage. For example, long narrow compartments of *E. globulus*, commonly used in agroforestry in lower rainfall regions, or compartments that include internal remnants of native vegetation, would be poor choices in parrot infested areas. These plantings would have considerably greater length of edge-to-area ratios than large block plantings, and would consequently expose relatively high numbers of trees to risk of parrot damage.

One of the major findings in this study is the strong relationship between damage and tree growth. For example, a study of three compartments from different plantations provided strong evidence that patterns of damage is not simply related to parrot abundance, or to the distribution of parrots surrounding these compartments, but is due to positive selection or preference by parrots for faster growing trees. In these cases, parrots evidently fly greater distances to reach faster growing trees, while ignoring closer trees with poor growth. Even within plots, parrots clearly demonstrate an ability to discriminate in selecting larger trees. However, the relationship between tree growth and damage was not evident in some compartments, whether due to a lack of variability in tree growth, uneven distribution of parrots around compartments, or to large compartment size relative to the parrot population. Indeed, in certain instances, large trees remained undamaged supposedly because parrots had not yet encountered them.

This examination of the distribution of parrot damage in even-aged plantations at different spatial scales has demonstrated that patterns of damage are related to three principle components (parrot abundance, tree growth and flight distance between parrot habitat trees and plantation trees). The study combines independent variables that best describe these factors to formulate a statistical model that explains over 70 % of the variation in damage among plots. This model may provide a useful framework around which management decisions can be made to reduce the risk of parrot damage. The modeling process has highlighted the need for more sensitive methods of assessing parrot abundance at plantation scale, and for including an additional variable indicating the availability and quality of alternative food at a prospective site (e.g. nectar flow, seed quality). Further development towards a predictive model might, for example, integrate tree productivity models based on site characteristics (e.g. PROMOD, Battaglia and Sands, 1997) with data on parrot abundance and plantation configurations. Greater effort could then be directed at controlling parrot numbers in areas predicted to be at high risk of damage, or conversely, plantation sites could be selected with low risk of damage.

Preliminary trials were set up in plantation areas with varied amounts of parrot damage, to assess whether the preference by parrots for faster growing trees was related to the foliar or stem nutrient status of trees (Chapter 3), but the results were not conclusive. The analysis was frustrated by finding only minor variations in tree foliar nutrient concentrations, despite large variations in both tree growth and parrot damage. This segment of the study, however, highlighted the possibility that any of a number of factors that limited tree growth might be related to damage.

During the course of the project, considerable research was directed at analysing the chemical composition of phloem sap in *E. globulus* and determining the range of values to be expected in trees growing on sites differing widely in availability of soil nutrients and water (Chapter 4). Seasonal variability was also assessed to see if any changes in phloem sap composition were consistent with seasonal patterns of parrot damage. Samples of phloem sap were collected from small incisions in the bark of young *E. globulus* and analysed for total sugars, free amino acids, organic acids and mineral composition. Total sugar concentrations in phloem sap were greatest from plantations in lower rainfall regions and when collected during the driest time of year, suggesting that the concentration of sugars in phloem sap varied with soil water availability. Total amino acid concentrations were shown to vary greatly, and were positively related to tree growth. This variability was reflected in very large differences in total C:N ratios in phloem sap (200 – 8200) that were negatively related to tree growth. It was then shown that trees least affected by nutritional and water stress, with narrower C:N ratios in the phloem sap, appeared to be most attractive to parrots.

It was tentatively concluded from the above that selection of trees by parrots might in fact be related to the nitrogen status of a tree, or, more specifically, to the current total amino acid concentration in its phloem sap. Concentrations of amino acids were also found to be greatest in upper stem sections where most trees were subject to damage. Conversely, concentrations of sugars tended to decline with stem height, so parrots would typically feed on'high nitrogen-low sugar' sap. These findings were consistent with field observations that damage was most severe in trees growing on sites with excessively high nitrogen.

Trees whose growth was currently not subject to major growth limitations were also found to bleed greater yields of phloem sap than stressed trees, and this effect was regarded as possibly of greater significance to parrots than simply total amino acid concentrations in sap. Indeed, parrots appeared to test stems before stripping the bark, possibly to locate trees with greater sap flow.

In addition to what is stated above, parrots damaged trees more severely in drier regions, and especially during the late summer and autumn, when concentrations of phloem sugars were greatest. But within these plantations, it still held that faster growing trees with lesser concentrations of sugars, and greater concentrations of amino acids, were most severely damaged. Greater intensity of damage to trees in drier regions can be partly explained by the greater numbers of parrots typically present in such regions (Chapter 2), while the increased amount of damage in autumn may be attributed to the generally low quality and quantity of available food for parrots at that time (Chapter 6).

Seasonal shortages of water can have large effects on growth of young *E. globulus* in the southwest of Western Australia. In Chapter 5 a new stable carbon isotope technique, involving analysis of phloem sap total carbon, was utilised to measure water stress in *E. globulus*, so this could be related to tree growth and other features in phloem sap composition. In addition, soil profiles were described to relate differences in soil water availability to tree performance and parrot damage. Phloem sap $\delta^{13}C$ proved to be a sensitive indicator of current water stress in even-aged young *E. globulus*, $\delta^{13}C$ values being greatest (least negative) in (a) regions of lowest rainfall, (b) in the driest months of autumn, and (c) in trees growing on shallow soils, or where there was a major impediment to root penetration.

This technique of assessing $\delta^{13}C$ of phloem translocate has certain advantages over other traditional measures of current water stress, and the results are easier to interpret than those based on foliar or wood $\delta^{13}C$ values used in earlier studies. Principle advantages are that (a) there is little variation with sampling time (diurnal) or sampling position on the stem, and (b) a measure is essentially made of discrimination of the most recently assimilated carbon product, and thus is representative of current physiological conditions. This technique has wide application in research and has been used in a number of recent studies (Pate and Arthur, 1998; Pate *et al.*, 1998; Pate and Arthur, 2000). One would also suggest it to have potential in the plantation industry for monitoring water stress and screening species and provenances for drought tolerance.

A close relationship was found in this study between phloem $\delta^{13}C$ values and sugar concentrations, with sugar concentrations increasing linearly with water stress, but related negatively to tree growth. On the other hand, concentrations of amino acids generally decreased markedly with increasing water stress and reduced nitrogen uptake. By contrast, trees of high foliar nitrogen status maintained greater concentrations of amino acids than expected in phloem sap during periods of moderate water stress, apparently due to remobilisation of soluble nitrogen reserves accumulated earlier in the season. As a general rule, trees of high nitrogen status accumulated both sugars and amino acids in phloem sap during autumn, thus contributing on both counts to increased attractiveness to parrots.

Two fertiliser trials were set up to test whether the composition of phloem sap in young *E. globulus* was sensitive to increased nutrient availability, and whether parrots could detect such changes (Chapter 6). These trials clearly demonstrated rapid increases in concentrations of amino acids in phloem sap in trees of low N status, following addition of N and NPK fertilisers, but these concentrations declined dramatically over the summer months as soils dried out. There were no consistent effects of fertiliser application on sugar concentrations or phloem sap $\delta^{13}C$ values.

These experiments showed fertiliser application did not increase the amount of damage caused by parrots. This was not surprising, since no growth response to fertiliser was evident in the time frame of the study, so presumably no visual cues would be available for parrots to discover that certain trees may be more nutritious than others. The other complication in this trial was that during spring, when concentrations of amino acids peaked, parrots had an abundant supply of grain in a nearby oat crop. Damage did increase after the oat crop was harvested, but by this time, concentrations of amino acids in phloem sap had decreased markedly alongside increasing water stress, and there was little variation in concentration among treatments.

The above trials illustrate the intrinsic difficulties one has in understanding the basis of food selectivity in wild animals. Tree nutrient status could be manipulated experimentally, but there was no evidence that parrots had detected such changes, or that these changes would coincide with parrots' current food requirements. These problems were exacerbated, of course, in the present study conducted over heterogenous mosaics of cleared and uncleared land, where parrots, due to their mobility, can select widely across a range of food resources..

Finally, a variety of aviary trials were conducted to demonstrate the ability of parrots to discriminate between different sucrose solutions and between adult and juvenile stems. These trials provided some evidence that parrots have this ability to select certain food resources over others. Patterns of damage in plantations might therefore not be random or solely a consequence of habitat factors, but the result of decided feeding preferences. Unfortunately, the demonstrated preference in captive parrots for sweeter sucrose solutions was, of course, not directly relevant to the situation in plantations, since volumes of solutions in the aviary trials were freely available, whereas in plantations, parrots have to work assiduously to obtain sap and in the process, appear to trade-off sugar concentration against volume of sap exuded. Nevertheless, the demonstration of preference for adult stems by parrots when in captivity more closely reflected the likely situation in plantations and indicated that some physical or perhaps chemical factors in the bark of juvenile *E. globulus* stems might deter parrots. The latter aspect is possibly worthy of further investigation.

Since completing this study, Chapman *et al.* (1999) have reported that a 'novel guild' of bird species, including lorikeets, cockatoos, and rosellas, were observed feeding on phloem sap exuding from sap-scars left by gliders on *E. resinifera* in north Queensland. They noted that crimson rosellas (*Platycercus elegans*) and sulphur-crested cockatoos (*Cacatua galerita*) appeared to pull at the bark surrounding the scars, possibly to keep the sap flowing. It appears that phloem sap is more widely used by Australian birds than previously thought.

Parrot damage in plantations of *E. globulus* is currently confined to the south-west of Western Australia, but it may not be long before other plantation areas, such as in south-eastern South Australia and western Victoria, are discovered as potential food sources by parrots. Indeed, there has already been one report of parrot damage in a young plantation near Penola, South Australia, identical to that so prevalent in Western Australia. One factor that may reduce the threat to these Eastern States plantations is the absence of cereal crops, which have undoubtedly contributed to the increase in parrot populations to pest status here in Western Australia.

In conclusion, this project has provided strong evidence that parrot damage in plantations of *E. globulus* is not randomly distributed, and that there are a number of factors contributing to the patterns of damage. Parrot abundance, habitat characteristics and tree growth factors need to be considered together to explain spatial variations in damage for a particular plantation. On the other hand, seasonal variations in damage are most likely a reflection of availability of alternative food resources for parrots. Preferential selection of faster growing trees from high nitrogen sites supports the view that parrots are deriving nutritional benefit from phloem sap and cambial tissues, and is fully consistent with "Plant Vigour" hypotheses for herbivory. While parrots appear to select stems with greater sugar and nitrogen content in phloem sap, it is quite likely that other, as yet undescribed, factors determine their choice of stems.

7.2 Future Research

During the course of this project I became aware of many issues that should be investigated to improve the understanding and control of parrot damage in eucalypt plantations. There is also a pressing need to evaluate the costs of this damage to the plantation industry. Some of these issues have already been mentioned, but it may be useful to list them here.

- Investigate the chemical composition of cambial tissues in stems of *E. globulus*, and how this varies with tree growth and nutritional status. Parrots scrape and possibly ingest the cambial tissues as well as phloem sap, so this material needs to be assessed for its nutritional value to parrots, compared with other food resources.
- Examine the effects of various nutritional and physiological stresses in *E. globulus* on cambium activity and cambium thickness. Growth stresses are likely to affect cambial activity and the amount of cambial material available to parrots. This may explain why trees affected by a range of growth stresses (nutrient deficiencies, drought, salinity or waterlogging) are less damaged than faster growing trees.

- Examine the effects of various nutritional and physiological stresses in *E. globulus* on bark thickness, ease of 'slippage', and other physical and chemical properties of bark. Parrots are likely to be deterred by bark that is difficult to remove or with high concentrations of secondary defence compounds.
- Determine whether the preference shown by parrots for adult stems is due to physical or chemical properties of stems. This may be useful for screening eucalypt species and provenances to find if there are any with stem or bark characteristics that may deter parrots.
- Determine whether parrots are prone to nutritional imbalances or deficiencies at certain times of year. It is not known whether parrots are seeking a specific nutrient or just need to supplement their energy intake.
- Determine characteristic density-dependent relationships for parrot damage in different areas. This information is needed to determine the level of population control required to reduce the level of damage in plantations.
- Examine the effect of availability of food resources and nesting hollows on parrot numbers and population dynamics, to assess whether these parameters could be manipulated to reduce parrot numbers and risk of damage.
- Determine the cost of parrot damage to industry. These costs include those associated with production losses, smaller piece size and increased costs of harvesting. Parrot damage also provides a point of entry for fungal diseases that may cause staining and wood rot, devaluing the quality of wood chips and potential sawlogs. A full assessment of these costs may provide greater justification of efforts to control the problem.

This project has defined the problem and provided some suggestions for reducing damage, but obviously a great deal of work is required before any sustainable solutions can be found.

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APPENDIX

Reprint of paper published in Oecologia in 1998, incorporating results of phloem studies at two plantations, Albany and Eulup, by Prof. John Pate and David Arthur, along with some of the results from the present study of phloem composition in three other plantations, Klopper, Naranbee and East from Chapters 4 and 5. These studies were done separately but the results were complimentary.

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Spatial and temporal variations in phloem sap composition of plantation-grown *Eucalyptus globulus*

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Abstract Spontaneous bleeding of sugar-rich sap from cambial-deep incisions in the bark of trunks was demonstrated for *Eucalyptus globulus* and other eucalypts across a range of localities and seasonal conditions in south-west Australia. High levels of sucrose and raffinose (up to 31% w/v total sugars) were present in the exudates, and upward and downward gradients in exudate sugar concentrations were recorded between samples obtained at different heights up trunks of *E. globulus*. The data indicated a phloem origin for the exudates, with source:sink pressure gradients driving translocation. Concentration ratios of sugars to amino acids were consistently lower in exudate from upper (distal) than basal regions of trunks, suggesting preferential partitioning of nitrogen upwards towards the trunk apex. A comparison of phloem and xylem sap composition from one plantation over a season showed nitrate in xylem but not phloem and substantial amounts of sodium, and high concentrations of chloride and sulphate relative to phosphate in xylem and phloem. Phloem sap sampled across a range of 29 contrasting plantations of *E. globulus* at peak stress (autumn) showed great inter-site variability in concentrations of amino acids, sulphur, sodium and certain trace elements and in C:N and Na:K ratios of sap. Carbon isotope ratios ($\delta^{13}C$) were strongly correlated with sugar concentrations of the sap samples from these and other plantations. Use of sap compositional attributes of phloem and $\delta^{13}C$ values of translocated carbon is suggested for assessing the current nutritional condition and water status of *E. globulus* plantings.

Key words Phloem bleeding · *Eucalyptus globulus* · Solute partitioning · Nutrient status · Water stress

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Introduction

Recent comprehensive treatments of the general ecophysiology (e.g. David et al. 1997; Osorio and Pereira 1994; Pereira et al. 1987) and nutrition (Adams 1996; Attiwill and Adams 1996) of eucalypts have highlighted the relevance of conventional physiological studies of growth, water relations and soil and foliar analyses of nutrients to forest performance and response to stress, but have provided little, if any, information on how carbon and nutrients are distributed in conducting systems of members of the genus.

The general composition of the nitrogen fraction of xylem sap of eucalypts has however been described for a few eucalypt species, and there have also been ecological studies of the relationship between xylem sap composition and herbivory (e.g. Adams et al. 1995; Journet 1980; Marsh and Adams 1995). For other tree genera, a number of authors have commented similarly on the utility of xylem sap analysis for diagnostic purposes, especially in studies of soil nutrient availability and tree nutrient status and growth potential (e.g. see Berger et al. 1994; Dambrine et al. 1995; Kazda and Weilgony 1988; Stark et al. 1984, 1989; Tonin et al. 1990; Walsh et al. 1984). Nevertheless, analyses of xylem sap alone are likely to constitute poor indicators of nutritional condition because of complications arising from (a) transient and long-term storage of xylem-delivered nutrients in mature parts of shoots and roots (e.g. see Jeschke and Pate 1995), (b) complex phloem-xylem and xylem-phloem transfers in leaves and stems and (c) fluctuations in xylem nutrient concentrations relating to soil water status and transpirational activity (e.g. Dambrine et al. 1995). In eucalypt seedlings, for example, Adams et al. (1995) have found that concentrations of reduced forms of nitrogen (e.g. glutamine) in xylem sap are negatively correlated with growth, implying that a number of other environmental factors might confound interpretations of nitrogen status based on xylem sap composition.

While the composition of phloem sap and the highly complex role of phloem translocation in nutrition are well documented for a number of herbaceous species (e.g. see Milburn 1975; Pate 1975, 1986; Van Die and Tammes 1975), there is little corresponding information for woody species. Collection of phloem sap from trees generally depends on the ability of trees to 'bleed' spontaneously from shallow incisions made in bark, as first demonstrated well over a century ago in the classic studies of Hartig (1860), and later, through the use of aphids and other phloem-feeding insects to obtain samples of 'sieve-tube exudate' (e.g. Fisher 1983; Lawton 1976; Raven 1983). Trees studied to date have been mostly northern hemisphere deciduous species (e.g. *Tilia*, *Fraxinus*, *Acer*, *Salix*) (see Ziegler 1975) but phloem or so called 'cambial' exudates of eucalypts have also been examined by Zimmerman and Ziegler (1975), Stewart et al. (1973) and Goldingay (1987), albeit solely in respect of sugar components of the sap.

Phloem sap sampling and analysis has undoubtedly provided critically important information towards the elucidation of seasonal, developmental and species-specific patterns of carbon and nutrient translocation in herbaceous species, and thereby aided understanding of how phloem and xylem partitioning processes of plants respond to changes in availabilities of nutrients, water and other resources (e.g. Pate and Jeschke 1995; Simpson 1986). We argue that the same potential exists for study of tree species, and report here our investigations of the phloem 'bleeding' phenomenon in *Eucalyptus globulus* and other eucalypts and the utility of analyses of such exudates in assessing nutritional status and translocatory performance of the species. The paper also introduces use of the carbon isotope ratio of phloem sap as a possible means for assessing water relations of trees under field conditions.

Methods

Collection of phloem sap from trunks of *E. globulus* and other eucalypts

Spontaneous bleeding of sugar-rich sap was first demonstrated using horizontal incisions made with a clean sharp razor blade to cambium depth (3–5 mm depending on bark thickness) in the trunks of 2- to-5-year-old plantation trees of Tasmanian blue gum (*E. globulus*) at Eulup, near Mount Barker, Western Australia in November 1995. The exudate was assumed to originate by pressure-induced mass outflow from cut sieve elements and is accordingly referred to as 'phloem sap' or 'phloem exudate'.

Where razor incisions penetrated deeper than the cambium, droplets of sap were quickly sucked back in, presumably escaping into the xylem. Conversely, shallow incisions to only 2 mm depth failed to bleed initially, but commenced to exude freely once the blade had been inserted a further 1 mm or so into the bark. Due to differences in bark thickness it was necessary to determine by trial and error the proper depth of cutting into bark of each set of study trees and thereby optimize success in sap sampling.

The standard procedure for collecting bulk phloem sap from *E. globulus* and plantations of other species of smooth-barked eucalypts, involved daytime (1100–1600 hours) sampling from groups of 20 randomly selected trees within a 0.5-ha area of plantation and collecting a 20-µl sample of sap from each tree using a series of three or four cuts to optimal depth spaced circumferentially around the trunk at 1.5 m above ground level. Sap droplets usually spurted out immediately from a cut, thereby enabling the required 20-µl volume of sap to be withdrawn into micropipettes within a few seconds of cutting. Sap samples were collected into 1.5-ml Eppendorf vials and quickly frozen in a portable refrigeration unit. Samples were then stored at −20°C to await analysis.

In a special investigation of 3- to 5-m trees of 1.5–2 years of age in the 1995 plantings at Eulup, phloem sap collections were made from series of cuts at 0.5-m intervals up a main trunk, commencing close to ground level and progressively upwards to a final cut as close as possible to the growing apex. Sets of samples obtained in this manner from single trees during the period September 1996 to February 1997 were used to study gradients in phloem concentrations of sugars and other solutes along trunks of the selected trees. At the same time, comparisons were made of bulked collections of sap from the bases and apical regions of matched sets of 20–30 trees from the same plantation at Eulup. It was then possible to determine whether the differences in composition of phloem exudates between upper and lower trunks observed for single trees applied generally to whole plantations.

Root xylem (tracheal) sap from *E. globulus* and comparisons with phloem sap composition over an annual cycle

This study, conducted in a plantation of uniform trees (1994 planting) at Eulup involved collection of xylem sap by mild vacuum extraction of freshly harvested lateral roots (1–3 cm diameter) at monthly intervals over the period May 1996 to June 1997, using the techniques described recently by Jeschke and Pate (1995) and Dawson and Pate (1996).

Five lateral roots were excavated from separate trees on each sampling date and samples bulked for analysis. Phloem sap of trunks at breast height was obtained simultaneously from a standard sample of 20 adjacent trees, using the techniques described above. The resulting analyses enabled variations in composition of xylem and phloem streams to be followed over an annual cycle of growth. Simultaneous measurements of main stem extension growth and increments in diameter at breast height (DBH) of trees in the plantation allowed changes in sap composition to be related to tree growth and rainfall, solar radiation and temperature for the year of study.

Analyses of phloem and xylem sap samples of *E. globulus* for organic solutes

Sugars, amino acids and organic acids of phloem sap were assayed using the HPLC-based system detailed by Pate et al. (1985). Routine field assays of sugar concentrations in phloem sap were also undertaken immediately upon collection using a temperature-compensated hand refractometer calibrated in the range 0–32% (w/v) sucrose. HPLC assays showed that more than 95% of the sugar fraction of the sap was composed of sucrose and raffinose, thus making it possible to use HPLC data on molar ratios of sucrose to raffinose to assess concentrations of total sap sugar from 'sucrose equivalent' values obtained in routine refractometer measurements.

By converting molar concentrations of all measured organic solutes to amounts of carbon and nitrogen (if present), the C:N weight ratio of translocated solutes was determined for each bulked phloem sap sample. This ratio was then employed as one of several indices of current nutritional status of the plantations from which the sap had been obtained. The same bulked phloem sap samples were also assayed for ^{13}C natural abundance ($\delta^{13}C$) of phloem carbon, using an ANCA combustion system (Europa Instruments) coupled to a SIRA9 VG Isogas Double-Inlet Mass Spectrometer. Precision of estimates of $\delta^{13}C$ between replicate 5-µl samples of phloem sap proved to be ±0.2. Differences in $\delta^{13}C$ values between phloem sap collected from a series of different plantations over the same time period (April 1996) were taken as tentative indices of current levels of water stress and instantaneous water use efficiencies of the plantations concerned (see Farquhar et al. 1988).

Analyses of phloem and xylem sap for mineral elements

Bulk samples of phloem sap obtained (a) during a single season (1996–1997) at the Eulup site and (b) across a range of 29 plantation sites sampled during autumn (1996) were assayed for the elements P, K, S, Na, Mg, Ca, Mn, Fe, Cu and Zn using an inductively coupled plasma (ICP) analytical system (McQuaker et al. 1979). Nitrate was measured by the automated method of Best (1976) and chloride by automatic colorimetry based on its reaction with mercuric thiocyanate in the presence of ferric nitrate (Technion, Auto Analyzer II Method 9970W). Element concentrations (µg ml^{-1}) and ratios between specific pairs of sap constituents (K:Na, amino acid N:P, amino acids sugars) were assessed for the relevant sap samples.

Results

Phloem bleeding in eucalypts

Sap of presumed phloem origin bled from cuts in bark of a range of main trunks of eucalypts in addition to the study species, *E. globulus*. Those producing collectable amounts of sap were *E. botryoides*, *E. cladocalyx*, *E. diversicolor*, *E. gratiae*, *E. leucoxylon*, *E. loxophleba* spp. *lissophloia*, *E. maculata*, *E. microcarpa*, *E. polita*, *E. polybractea*, *E. saligna*, *E. sideroxylon*, *E. vegrandis*, *E. viminalis*. In all cases, except for *E. globulus*, evidence for a phloem origin of the exudate was based solely on refractometer or HPLC measurements of high (18–35% w/v) concentrations of sugars.

E. globulus proved to be a particularly prolific and consistent phloem bleeder provided that sampling was conducted on relatively young trees with trunks of 1.5–10 cm diameter. Exudation continued for 5–30 s after cutting and then ceased abruptly. It was usual for 90% or more of the individuals in 1.5- to 3-year-old plantations to bleed adequately on cutting main trunks at the standard height of 1.5 m. Failure to bleed was rare but encountered occasionally when severely water stressed plantations were sampled during very hot afternoons of late summer and autumn. Even then, the same trees produced sap when sampled after recovery of water stress the following evening.

Directionality of exudation in *E. globulus* was deduced in profusely bleeding trees using a technique in which horizontally oriented 'windows' (4 × 4 cm) of bark were excised down to cambium level. In virtually all cases, windows sited on regions of trunk above the main foliar zone on a canopy maintained bleeding only from their basipetal (lower) faces, whereas stations situated below the main leaf canopy bled only from their acropetally directed (upper) faces. We believe that in either case, the face from which sap exudation was protracted represented that whose translocation was being maintained by mass outflow from sieve elements still receiving sugar from source foliage. In other words the 'bleeding face' of each bark window invariably represented the more concentrated 'source' side of a decreasing gradient in sugar concentration.

Phloem sugar concentration gradients along trunks as evidence of bidirectional translocation in *E. globulus*

Daytime sampling of relatively unstressed 5- to 6-m-high (1995 planting) trees at Eulup in the period September 1996 to February 1997 showed marked gradients in phloem sap total sugar concentration at different trunk heights in all trees studied. Data for daytime (1100–1400 hours) measurements on groups ($n = 25$) of uniform trees in September and November are shown in Fig. 1. The values obtained, expressed in Fig. 1 as means with standard errors of phloem sugar concentrations at different positions on the trees sampled, are fully consistent with opposingly directed upward and downward pressure flow gradients transporting sugar from a central loading region comprising leafy regions of the shoot canopy.

Evidence of differential partitioning of N between upward- and downward-moving translocation streams

Having indicated that trees typically exhibited bidirectional patterns of photoassimilate translocation, we then tested whether concentration of nitrogenous solutes relative to sugars changed in a manner indicating preferential loading of N onto either the upward or downward translocation stream. The single 4-m-high tree studied at Eulup in September 1996 (Fig. 2) displayed typical downward and upward gradients of decreasing

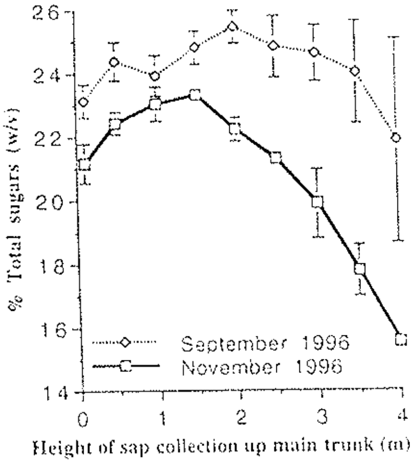


Fig. 1 Gradients in concentration of total sugars in phloem sap collected at different heights up the main trunks of unstressed 5-m-high trees of *Eucalyptus globulus* sampled on two occasions at Eulup, Western Australia. Data are expressed as means with standard errors for the group of 25 trees sampled. All trees were sampled in daytime (1100–1400 hours) using the sap collection procedure described in the text

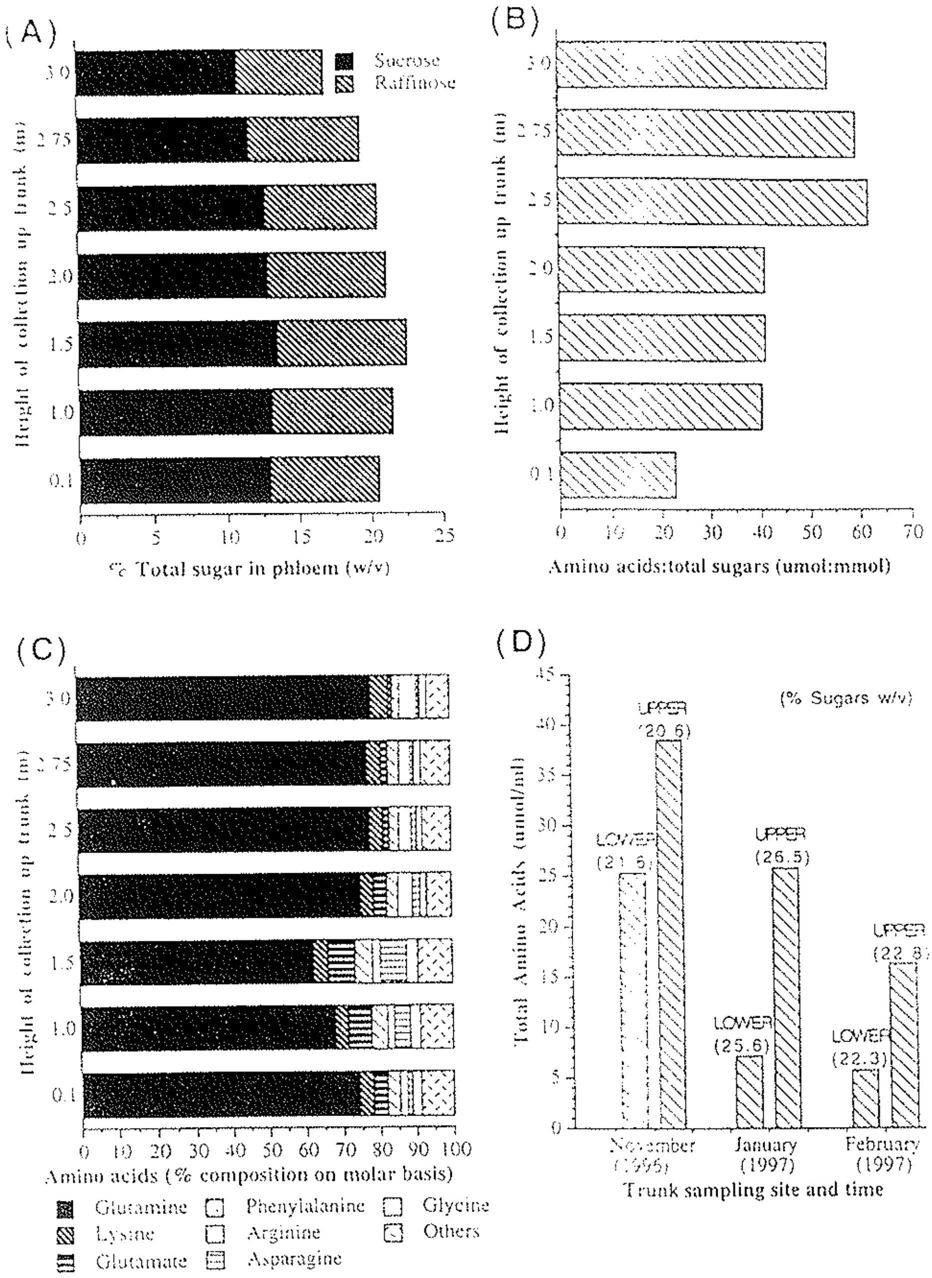


Fig. 2 Compositional features of phloem sap collected from trunks of uniform 2-year-old trees of *E. globulus* in rainfed plantation culture at Eulup, Western Australia. **A–C** Data for raffinose and sucrose concentrations (**A**), amino acid:sugar molar ratio (**B**) and amino acid composition (**C**) of phloem sap collected at different heights up the trunk of a 3.5-m-tree in September 1996. **D** Concentrations of total amino acids, and total sugars (*values in parentheses*) in bulk samples of phloem sap collected from the upper and lower regions of trees of the same plantation. A set of 20 uniform trees was sampled on each specified date

concentration of sugars, with a peak concentration value at the presumed main loading zone 1.5 m above ground level (Fig. 2A). As observed for many other trees, ratios of raffinose to sucrose did not change appreciably as translocate moved up or down the trunk. However, molar ratios of total amino acids to total sugars (Fig. 2B) proved to be several times greater at the highest than the lowest sampling points on the trunk. Since percentage composition of the amino fraction of the upper stream did not differ markedly from that of the lower stream (Fig. 2C), it was concluded that this enrichment of the acropetal relative to the basipetal phloem stream must have been due to preferential upwardly directed loading of all major translocated nitrogenous compounds. However, when expressed in terms of differences in the total N and associated C:N ratios of these upper and lower streams, implementation of the enrichment process was accomplished mostly by loading of the major solute glutamine.

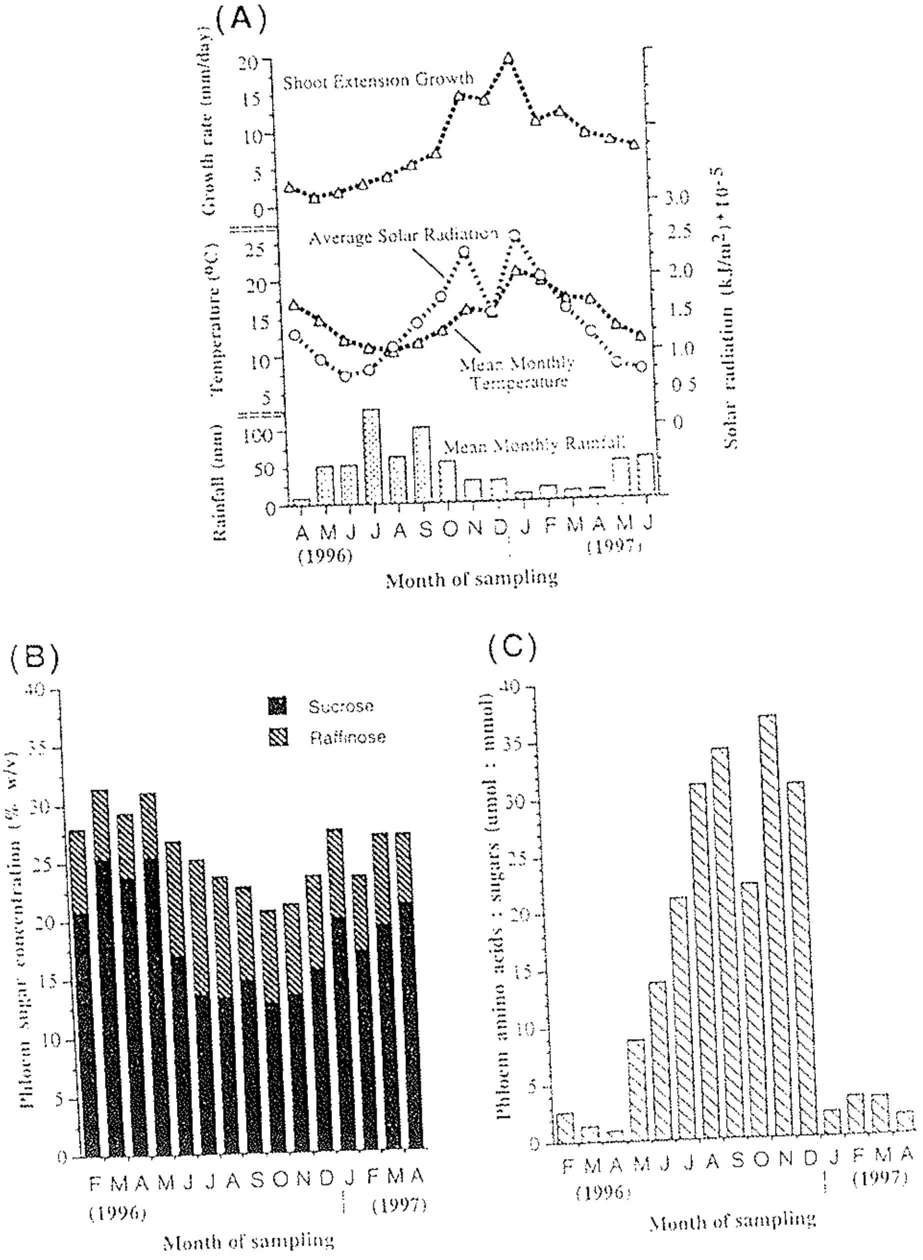
The phenomenon described above (Fig. 2A–C) for single trees applied also to the amino acid:total sugar balance of bulked samples of phloem sap collected from upper regions (above 3.5 m) and basal regions (below 0.5 m) of trunks of 20 trees of the same plantation at Eulup studied during November 1996 and January and February 1997. In all cases (see Fig. 2D), concentrations of total amino acid (N basis) were greater in upper than lower phloem samples, despite only small differences in phloem total sugars (values in parentheses in Fig. 2D) in the phloem streams within and between sampling times.

Seasonal variations in sugar concentrations in phloem and amino acid composition and mineral ion balance of phloem and xylem sap at a single site

This comparative study was conducted in the 1994 planting of Eulup over the period February 1996 through June 1997. Rates of apical extension of trunks during the study period (Fig. 3A) are shown to be positively related to temperature and solar radiation but inversely related to rainfall received during the season. Concentrations of total sugars (Fig. 3B) in phloem collected at 1.5 m height were generally greater in the hot and dry months of summer and early autumn (January to April) than during the wetter cooler months of May to December. Highest concentrations of phloem amino acids occurred in spring through to early summer (September to December) when the tree growth rate was increasing and mineral N likely to be readily available from the soil. A several-fold greater ratio of amino acids to total sugars in phloem sap was accordingly recorded during this period (Fig. 3C) than in the dry period between January and April.

Glutamine was the major N-containing solute of phloem at all times of sampling (Fig. 4A), with some evidence of highest proportional concentrations of the compound when the total N load of the phloem was greatest in spring and early summer. Other compounds typically present in appreciable quantities were glutamate, aspartate, phenylalanine, glycine, alanine and serine. Minor compounds. ('others', Figs. 2C, 4)

Fig. 3 Seasonal study of trunk extension growth in relation to climatic variables (A), and corresponding monthly variations in phloem sap composition of sugar components (B) and amino acid:sugar balance (C) in *E. globulus* trees from the 1994 planting at Eulup, Western Australia



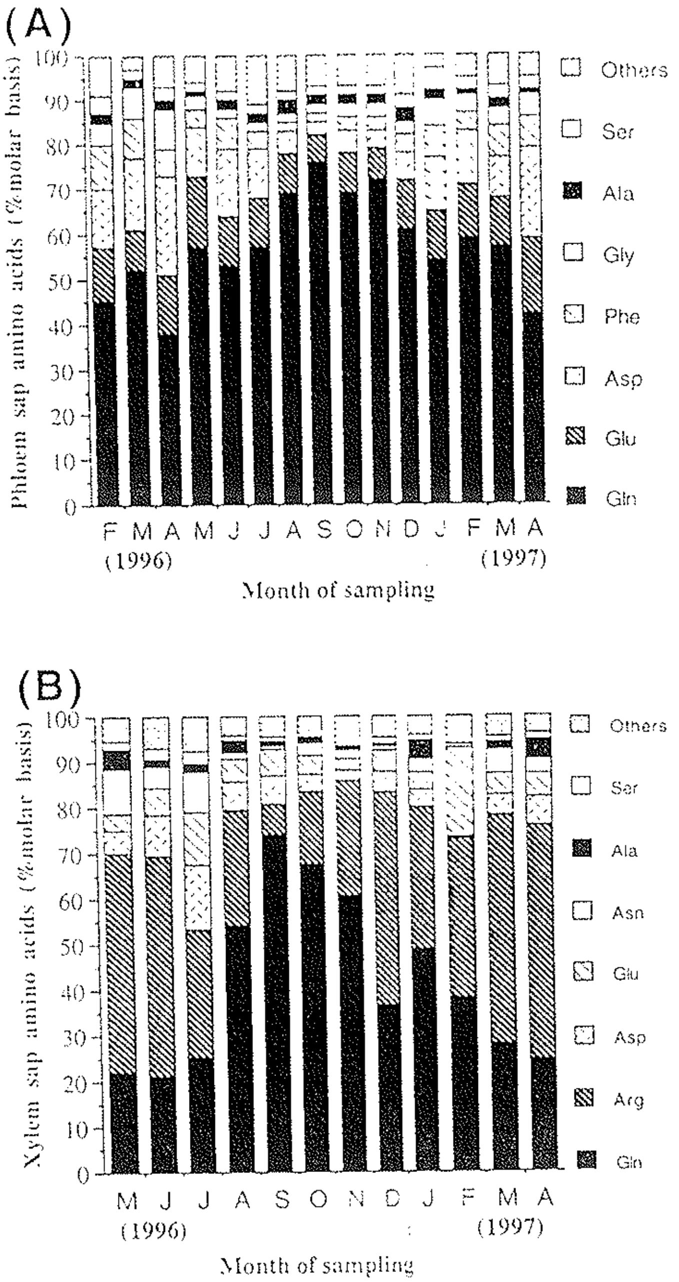


Fig. 4 Monthly variations in percentage amino acid composition of trunk phloem sap (A), and lateral root xylem (tracheal) sap (B) collected from trees of *E. globulus* from the 1994 planting at Eulup featured in Fig. 3

Table 1 Mean concentrations of major inorganic components of phloem and xylem sap collected at monthly intervals over the period May 1996 and June 1997 from a plantation of uniform trees (1994 planting) at Eulup, Mount. Barker, Western Australia

Component	Mean (±SE) concentration (mg l^{-1})	
	Phloem sap	Xylem sap
K	1136 ± 15	128 ± 23
Ca	9.8 ± 0.5	43.2 ± 8.9
Mg	50.7 ± 6.2	16.5 ± 3.7
Na	241 ± 30	27.1 ± 5.5
Total S	59.3 ± 4.1	29.3 ± 18.4
Total P	58.3 ± 8.3	5.67 ± 1.74
Cl	76.7 ± 10.5	58.3 ± 13.6
NO_3	Trace	1.58 ± 0.27

included asparagine, threonine, proline, citrulline, valine, cystine, methionine, isoleucine, leucine, tyrosine, histidine, lysine and arginine. The comparable picture for xylem amino acids (Fig. 4B) differed principally in showing generally much greater proportional amounts of arginine than in phloem. Indeed, xylem concentrations of arginine on a molar basis exceeded those of glutamine at certain times (April, May, December samples), and when this was not the case, rated second in order of abundance to glutamine. Asparagine was also at proportionately higher concentration in xylem than phloem.

Analyses of the mineral composition of phloem and xylem sap collected across a growing season from the Eulup site (Table 1) showed potassium to dominate the cationic component of both streams. Concentrations of chloride were consistently very high relative to phosphate, sulphate and sodium. Nitrate was transiently present at low concentration in xylem but never detected in phloem. As found generally for other species, concentrations of calcium were less in phloem than xylem.

Variations in phloem sap composition across plantations of *E. globulus* sampled simultaneously

This study, examining variations in mean concentrations of a range of organic and inorganic components of phloem sap across 29 contrasting plantation sites, was undertaken in autumn (April 1996) when trees were likely to be experiencing peak water stress and minimal availability of nutrients from generally dry soil. Twenty-nine separate 2-year-old plantations were examined, encompassing as wide as possible a range of the climatic conditions and soil types likely to be experienced in plantations of *E. globulus* in south-western Australia.

Data for means and ranges of concentration values for solutes (Table 2) showed a relatively small (1.5-fold) range in total sugar (raffinose and sucrose) levels across the sites (539–824 μmol ml^{-1}) compared with a much greater (28-fold) range in levels of total amino acids (0.7–20 μmol ml^{-1}) and an almost 4-fold concentration range for total organic acids (2.6–9.9 μmol ml^{-1}). Major organic acids in order of abundance on a molar basis were malic, citric, fumaric and shikimic acids. As shown for the seasonal study at Eulup (Fig. 4B), the amino acid fractions of phloem sap from all sites (data not shown) were consistently dominated by glutamine with lesser amounts of glutamate, aspartate, glycine, alanine, phenylalanine and lysine. Concentrations of P, K, Mg and Ca (3- to 6-fold variations for all four elements) were less variable between sites than S, Na and the trace elements

Table 2 Concentrations of organic and inorganic solutes and concentration ratios of selected pairs of solutes in phloem sap obtained from 29, 2-year-old plantations of *Eucalyptus globulus* sampled in April 1996. The sites selected were considered broadly representative of the range of climatic and edaphic conditions in south-western Australia. (See also Table 3 for C:N weight ratios of the same sap samples)

Component	Mean	Range
Amino acids (μmol ml^{-1})	11.0	0.7–20.2
Organic acids (μmol ml^{-1})	5.03	2.6–9.9
Total sugars (μmol ml^{-1})	674	539–824
K (μg ml^{-1})	1321	340–1985
Na (μg ml^{-1})	321	85–945
Mg (μg ml^{-1})	63	35–89
Ca (μg ml^{-1})	11	5.3–20
P (μg ml^{-1})	55	35–130
S (μg ml^{-1})	193	40–488
Mn (μg ml^{-1})	3.4	1.0–13.8
Fe (μg ml^{-1})	5.4	2.0–19.3
Cu (μg ml^{-1})	0.5	0.3–3.9
Zn (μg ml^{-1})	1.3	0.3–9.2
Ratio		
Raffinose:sucrose (μg:μg)	0.29	0.09–0.54
Amino acid:total sugars (μmol:mmol)	16.2	1.1–30.6
Amino acid N:P (μg:μg)	4.5	0.15–11.39
K:Na (μg:μg)	[illegible]	0.4–20.7
K:P (μg:μg)	24	37.0

Mn, Fe, Ca and Zn (10- to 31-fold variation for all six listed elements).

Ratios between selected pairs of solutes are also given in Table 2. The mean molar ratio of raffinose to sucrose in the sap samples studied was 0.29 ± 0.10 (SD), the ratio of total amino acids to total sugars (μmol:mmol) showed a mean value of 16 with a large 28-fold range of values. This was taken as evidence of wide variations in translocatory capacity for N relative to sugars. In similar fashion, the massive 57-fold variation in N:P (μg:μg) ratios implied extremely wide ranging differences in relative intensities of translocation of these two nutrients between study sites. Equivalently great variation applied to the 50-fold range in K:Na ratios, in this case presumably reflecting great variability in K and Na status of soils at the study sites and a corresponding flexibility of the species in substituting Na^+ for K^+ to maintain the monovalent cation status of phloem sap under low K^+ conditions. The final ratio listed [K:P (w/w)] showed a mean of 24 and a 14-fold range spanning 2.6–37.

Data for phloem concentrations of all organic acids, amino acids and sugars are used in Table 3 to compute C:N weight ratios for the phloem sap samples collected at each site. The values show a very large (almost

Table 3 Concentrations of C and N in organic fractions of phloem sap obtained in April 1966 from a series of 29 2-year-old plantations of *E. globulus* spanning a wide range of climatic and edaphic conditions in south-western Australia

Site no.	Amino acid N (μg/ml)	Amino acid C (μg/ml)	Sucrose C (mg/ml)	Raffinose C (mg/ml)	Organic acids C (μg/ml)	Total C (mg/ml)	C/N weight ratio
1	472	1120	77.5	37.9	264.0	117	247
2	470	1116	71.2	33.9	295.8	106	226
3	48	152	103.5	22.7	501.4	129	2637
4	315	773	77.9	28.5	349.1	108	341
5	186	493	91.7	28.8	254.2	121	651
6	290	688	72.2	38.7	294.0	112	386
7	116	307	88.1	24.3	289.3	113	972
8	132	342	77.6	34.3	210.2	113	851
9	192	519	79.9	34.6	244.1	115	599
10	90	235	85.8	28.9	307.2	115	1282
11	51	155	83.9	29.7	441.1	114	2265
12	171	452	74.5	34.1	303.4	109	639
13	487	1154	76.4	37.8	223.6	116	237
14	424	996	77.1	36.9	221.2	115	272
15	132	316	68.4	36.7	282.6	106	798
16	171	424	73.7	32.6	169.9	107	626
17	526	1226	68.7	41.6	333.5	112	213
18	296	702	61.5	34.5	177.6	97	328
19	387	925	62.2	34.1	257.1	97	252
20	20	49	52.7	37.5	185.3	90	4599
21	428	1006	64.8	36.9	273.3	103	241
22	434	999	54.5	44.5	243.5	100	231
23	140	325	63.5	33.4	315.0	98	699
24	495	1154	75.7	21.6	342.7	99	200
25	399	936	76.3	22.3	268.5	100	250
26	439	1032	76.8	22.5	247.0	100	229
27	269	639	87.7	21.9	269.7	111	411
28	12	29	73.8	25.7	142.1	100	8199
29	33	83	90.4	23.6	333.1	114	3487
Mean	263	632	75.5	31.8	277.6	108	1116

40-fold) range (213–8333) around a mean of 1117, reinforcing earlier conclusions regarding the ability of *E. globulus* to load phloem with highly variable amounts of amino acids relative to sugars and other components providing C, but not N, to the translocate.

Phloem sugar concentrations and $\delta^{13}C$ of phloem carbon as possible indices of site-specific water stress in plantations of *E. globulus*

These exploratory analyses involved inter-site comparisons of composition of bulk phloem sap of a broad range of plantations sampled in autumn (April 1996) and close to or coinciding with maximum seasonal water stress. The study involved sampling ten replicate trees from 37, 2-year-old plantings in the vicinity of Northcliffe, Boyup Brook, Chowerup, Mount Barker and Albany. The sites encompassed a broad range of growth rates (as assessed by height or basal area) and equally great inter-site variations in soil type and mean annual rainfall (600–1400 mm).

The total concentration of sugar in phloem (%w/v) proved to be closely correlated ($R^2 = 0.69$) with $\delta^{13}C$ of phloem carbon (Fig. 5A). At one extreme were data for the highly stressed trees of the Eulup site, where phloem sugar concentrations exceeded 30% w/v and $\delta^{13}C$ values lay within the unusually high (less negative) range from −19 to −20. Values of this order would be expected to be associated with extreme shortages of water and correspondingly good instantaneous water use efficiency. At the other extreme were the rapidly growing trees at Albany, where irrigation through summer involved application of waste water from a sewage treatment plant. Phloem sugar concentrations in this case were very low [20–22% (w/v)] and phloem carbon well depleted in ^{13}C ($\delta^{13}C$ values of −26 to −27), both measurements indicative of relatively unstressed conditions.

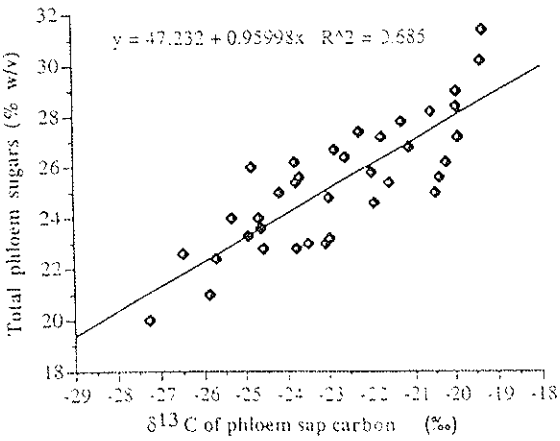


Fig. 5 Correlation plots of phloem sugar concentration and $\delta^{13}C$ signal of phloem sap carbon of *E. globulus* for 29 2-year plantations sampled at a series of sites in south-west Australia in autumn 1996 at or close to maximum seasonal water stress

Discussion

As far as we are aware, the first published record of the composition of phloem exudates of *Eucalyptus* spp. is that of Zimmermann and Ziegler (1975) in their comprehensive analyses of sieve tube exudates of trunks using the classic bark incision technique of Hartig (1860). Their survey, covering some 500 tree species from about 100 plant families, involved semi-quantitative assays for sugars and sugar alcohols, using paper chromatography to separate and identify compounds. Four species of *Eucalyptus* were listed by Zimmerman and Ziegler (1975), and raffinose was identified as the major exudate sugar accompanying sucrose in all species. With 16 of 19 other taxa of Myrtaceae also recording positive for raffinose, Ziegler (1975) grouped the family alongside members from 14 other families as specializing in phloem transport of sucrose and raffinose.

Raffinose has also been identified as a major phloem ('cambial sap') component of *E. regnans* by Stewart et al. 1973, and other observations on sap flow and sugar content of eucalypt phloem exudates are available from the studies of Smith (1982) and Goldingay (1987) on the feeding behaviour of the Australian marsupial gliders, *Petaurus australis* and *P. brevipes*. These animals initiate phloem bleeding by making incisions in bark with their teeth and ingestion of exudate is rated as a significant dietary component during certain times of the year. Goldingay (1987) concluded that trees highly favoured and defended by *P. australis* might possess special exudation characteristics facilitating prolonged exploitation as a food source.

Our observations involving razor blade incisions to cambium depth in bark of trunks of *E. globulus* and a range of other smooth-barked eucalypts suggest a general propensity for phloem bleeding within the genus. Just as demonstrated earlier by Huber et al. 1937, Hammel 1968 and Zimmerman 1971 for deciduous trees such as *Quercus* and *Fraxinus*, concentration gradients of sugar in phloem exudates collected at different heights up trunks of *E. globulus* are strongly indicative of source:sink pressure gradients driving translocation. Furthermore, the young trees we examined showed both downward and upward gradients in sugar concentration from a maximum point emanating from the major leafy region of the canopy. This finding, together with evidence of directionality of bleeding from faces of cut-out 'windows' in bark, indicates that upper parts of the foliar canopy of young trees typically supply photosynthates to the trunk apex, while lower regions of the canopy principally serve the root system.

Our standard phloem-bleeding technique, which collects sap at a relatively low height (1.5 m) from trees

4–5 m tall, supposedly samples the downward-moving translocation stream, so the general compositional features recorded in this paper between sites or across a season at a single site presumably reflect the nature of the translocate specifically supplied to lower trunk and roots.

Concentration ratios of total amino acids to sugars in samples of phloem sap obtained from upper near-apical parts and lower bole regions of trunks of *E. globulus* provide consistent evidence of preferential loading of amino acids onto acropetal as opposed to basipetal streams of translocate. For example, using data shown in Fig. 2, C:N weight ratios of C-rich lower phloem streams were 2.4 times greater on average than those of corresponding N-rich upward moving streams. Phloem partitioning of C and N in trunks of *E. globulus* is thus remarkably similar to that described for stems of the herbaceous legume *Lupinus albus* by Pate and Layzell (1981) or for other herbaceous species by Pate and Jeschke 1995. Empirical models of C and N flows in the latter non-woody species have indicated that stems engage in complex series of xylem-to-xylem and xylem-to-phloem transfers which collectively restrict the cycling of N through lower leaves and back to the root in the phloem, while greatly enriching with N the phloem stream moving from upper leaves to the shoot apex (see Pate 1986). Nodal regions of the stems of the abovementioned herbaceous species and, more specifically, the transfer cells which they contain (Pate and Gunning 1972) are held responsible for these enrichment or depletion processes of N, and it would be particularly interesting to determine what corresponding structural and functional attributes perpetuate a similarly strong bias towards upward translocation of N in the much more complex system afforded by a woody multi-branched tree such as *E. globulus*.

Since this study comparing the composition of phloem sap of bases and tops of trunks was completed, similar measurements have been made in other plantations and seasons. The data show that the marked differential between the two streams with respect to N and amino acid:sugar balance reported in this paper is not always evident. It would thus appear that the enrichment of upward-flowing phloem with N may be downtuned or almost completely turned off under certain circumstances. This phenomenon is currently being further investigated in relation to plantation growth under different nitrogen regimes.

Comparisons of amino acid composition of phloem and xylem sap of *E. globulus* presented in this paper show high proportional amounts of arginine in xylem but not in phloem. Absence of this amino acid has also been reported for phloem sap of both *Lupinus* (Pate et al. 1979) and *Banksia* (Jeschke and Pate 1995), while evidence of a predominant role for the compound in xylem transport and storage of N has been reported for a number of woody species, including *E. globulus*, *E. regnans* and *E. nitens* (Adams 1995) and apple (see Pate 1980). In similar fashion, seedlings of *E. globulus*, *E. regnans* and *E. nitens* accumulate arginine in old but not young foliage (Adams et al. 1995). Further studies of seasonal fluxes of N in xylem and phloem are clearly required before the dynamics of uptake, temporary storage and mobilization of arginine and other forms of N can be properly appreciated in eucalypts.

The studies of herbaceous species alluded to above have also provided evidence that the lower nodal regions of the stem engaging in intervascular exchanges of N can also be equally actively committed to selective transfer of Na back to roots coincident with preferential retention and upward flow of K within shoots (see situation summarized for the salt-tolerant genera *Ricinus* and *Hordeum* by Pate and Jeschke 1995). It remains to be seen whether similar selective capacities exist in *E. globulus*, and whether such discrimination is better displayed in other eucalypts more tolerant of saline situations. It would be particularly valuable in this connection to compare xylem sap composition with that of phloem sap of tops and bases of trunks to monitor how current patterns of partitioning of specific minerals within shoots respond to nutritional deficiencies or imbalances within the tree.

The data comparing the mineral composition of phloem and xylem sap of *E. globulus* through a season at the one site (Table 1) are remarkably similar to those recorded by Jeschke and Pate (1995) for the native south-western Australian tree *Banksia prionotes*. Compared to data available for agricultural species, phloem and xylem of both *Banksia* and *Eucalyptus* contain unusually high levels of chloride and sulphate relative to phosphate, and substantial amounts of sodium relative to potassium. As suggested for *Banksia* by Pate and Jeschke (1993) and Jeschke and Pate (1995), the data for *E. globulus* indicate an inherent capacity to substitute non-limiting nutrients such as chloride, sulphate and sodium for phosphorus and potassium when loading transport streams under conditions in which the latter two key nutrients are severely limited.

Corresponding data for phloem sap mineral composition across a wide range of sites at the one time of sampling (Table 2) show enormous ranges in concentrations of a number of minerals and organic solutes. Greater variability across the data set is evident in concentrations of N, S, Na, Mn, Fe, Ca and Zn and in ratios of amino acids:sugars, amino acids:P, C:N and K:Na than in concentrations of other components such as P, K, Mg and sugars. We suggest that, if this data base were extended to include much greater numbers of sites and times of sampling, the phloem sampling technique might prove to be a useful means for rapid assessment of tree nutrient status, providing information for future fertilizer management options for plantations.

This paper presents evidence of a surprisingly strong positive correlation between δ^{13}C and concentrations of total sugars in phloem sap samples collected across a range of plantations of *E. globulus*. The physiological basis for this close relationship is not yet clear, but it is suggested that one or both quantities might be used instructively in the future for monitoring water stress and instantaneous water use efficiencies in plantations of the species. A companion paper (Pate et al. 1998) explores these issues.

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