Surviving the fire-trap: resprouting and carbohydrate partitioning of *Acacia karroo* after topkill

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Abstract

There is a growing recognition of the role of fires in controlling the structure and density of trees and shrubs in savannas. With a global trend towards tree densification in rangelands and savannas, fire is viewed as a tool that can slow or even reverse this transition. Although fires often cause stem death, or topkill, most savanna trees resprout from the surviving rootstock. This study investigated the carbon allocation patterns that enable the spindle form of *Acacia karroo* found in the Hluhluwe-iMfolozi Park, KwaZulu-Natal, to survive repeated and frequent topkill. The central hypotheses were that resprouting is dependent on root carbohydrate reserves and that very frequent topkill would progressively lower the carbohydrate reserves of a plant and eventually cause mortality.

The root starch concentration of resprouting *A. karroo* saplings decreased by 75% during the first 6 months after topkill but recovered to pre-fire levels after an additional 6 months if the plants were protected from defoliation. The root starch concentration of unburnt *A. karroo* saplings also decreased over the growing season to 50% of the annual maximum concentration that occurs late in the dry season. The aboveground growth rates of saplings in the second year after two consecutive annual topkill events were not significantly different from the growth rate in the first year. ^{14}C labelling experiments showed that root reserves are remobilised and incorporated into the tissue of resprouting shoots. Using a photosynthesis model it was estimated that for nearly 4 months after topkill, the amount of carbon incorporated into shoot biomass exceeded the amount of carbon that had been assimilated since topkill. However, after 12 months, the amount of carbon assimilated since topkill far exceeded what had been incorporated into shoot biomass and what was available from root carbohydrate reserves. For plants that had a similar pre-fire size, the leaf area of resprouting plants recovered to match that of unburnt plants within 4 to 5 months after topkill. Aboveground growth of resprouting plants occurred largely in the first few months of the wet season, whereas photosynthetic rates remained high into the start of the dry season.

I propose that root starch reserves supplement the rapid recovery of leaf area of *A. karroo* at the start of the first growing season after topkill. Once the leaf area of the plants has recovered, the plants have the photosynthetic capacity to replenish any spent reserves over the remainder of the wet season and into the dry season. In this study, plants were topkilled late in the dry season when reserves are at a maximum. Topkill at other times of the year when root starch concentrations are low might have a more lasting effect on aboveground recovery and root starch replenishment and this needs to be investigated further.

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Chapter 1 : Introduction

Surviving the fire-trap

Savannas are characterised by a continuous grass layer and a discontinuous tree layer. Savannas are amongst the most fire prone ecosystems in the world (Mouillot & Field 2005). In mesic savannas fire-intervals can be as short as 1 to 3 years (Frost & Robertson 1985; Bond & Midgley 2000; Hoffmann & Solbrig 2003). The potential for frequent fires in savannas results from the coincidence of a continuous highly flammable dormant grass sward and hot dry periods in the winter months (D'Antonio & Vitousek 1992; Mouillot & Field 2005). Fires in savannas often kill the aboveground parts of short trees and shrubs, but total plant mortality is usually low (Glitzenstein *et al.* 1995; Williams *et al.* 1999) as the plants resprout from the surviving root tissue (Frost & Robertson 1987; Trollope 1984). The death of aboveground parts that leave a surviving root is referred to as topkill. Once trees grow to a certain height (2–3m) they become less vulnerable to topkill (Trollope 1984). However, the frequent fires in mesic savannas allow few opportunities for juvenile trees to reach such a height (Bond & van Wilgen 1996; Higgins *et al.* 2000). Many savanna trees survive for decades in a stunted, often multi-stemmed, form by resprouting time after time from the surviving rootstock but unable to escape the so-called fire-trap (Fig. 1.1) (Bond & van Wilgen 1996; Gignoux *et al.* 1997).

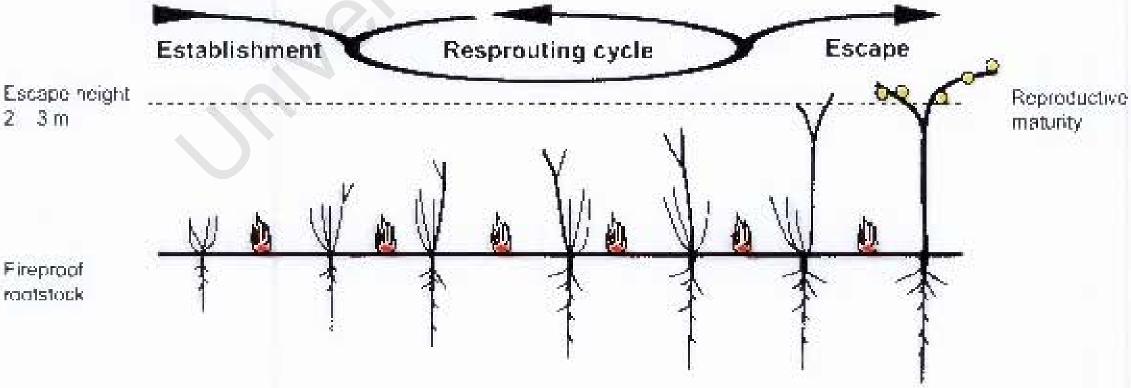

Fig. 1.1. The resprouting cycle of savanna trees as described by Bond and van Wilgen (1996) and Gignoux *et al.* 1997. The short interval between fires in mesic savannas allow few opportunities for saplings to grow tall enough to avoid topkill. Plants can survive for decades as stunted individuals surviving multiple topkill events by resprouting from the fireproof rootstock. Immediately after topkill, many coppice shoots emerge but with time one of the shoots attains apical dominance and grows faster than the other shoots. As the root structure of the plant grows, it is able to store more carbohydrate reserves, access more resources, and thus support more vigorous resprouting after topkill. If there is a sufficient break in the fire cycle or a series of cool fires that do not cause complete topkill the plants escape the fire-trap. Often the trees will only become reproductive once they have escaped the fire-trap.

The carbon cycle of resprouting plants

The accumulation of carbohydrate reserves, particularly starch, is often seen as a key trait of resprouting species (Pate 1990; Knox & Clarke 2005). Immediately after topkill, carbohydrate reserves would supply the carbon necessary for new growth and maintenance of surviving tissues until these demands could be met by concurrent photosynthesis (Chapin *et al.* 1990; Kobe 1997). A hypothetical model of reserve mobilisation and replenishment is shown in Fig. 1.2. After topkill, a plant has no photosynthetic tissue and carbohydrates required for growth and maintenance are supplied by carbohydrates stored in the root (between A and B in Fig. 1.2). As the leaf area on the new shoots increases so does the net photosynthetic gain of the plant. At some point photosynthetic gain is sufficient to meet the growth and maintenance costs of the plants and the reliance on reserves ends (B in Fig. 1.2). Once photosynthetic gain exceeds growth and maintenance costs, this excess is then used to replenish the spent reserves (B to C in Fig. 1.2). If plants are to survive a series of very frequent fires they either need to have a reserve pool that is large enough to support the growth of multiple generations of resprouting shoots or they need to replenish reserves between the fires (Bellingham & Sparrow 2000; Iwasa & Kubo 1997).

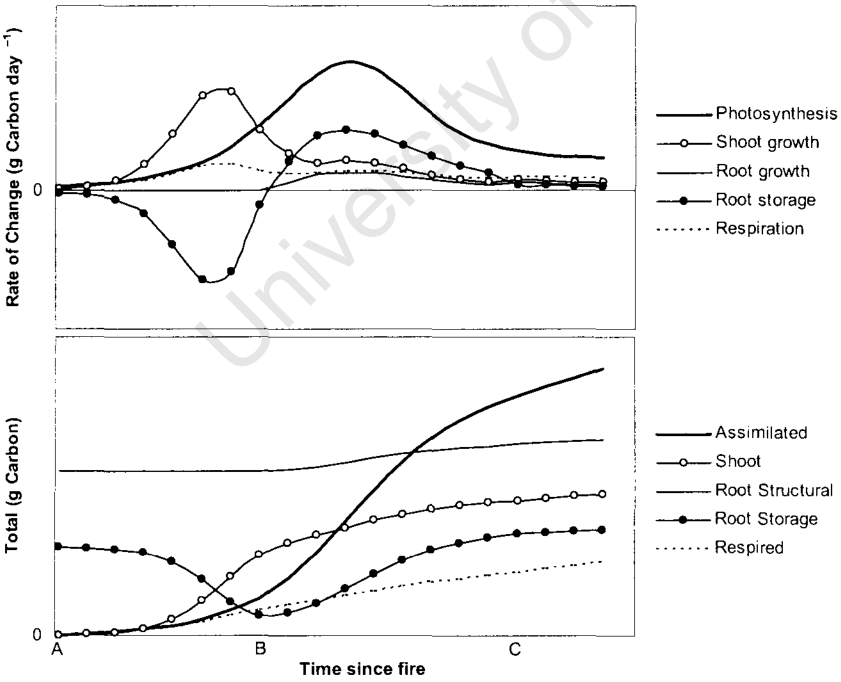

Fig. 1.2. The carbon budget of resprouting trees. Immediately after topkill, the carbon required for shoot growth, including leaf area renewal, and tissue maintenance exceeds that available from concurrent photosynthesis (A to B). Root reserves are mobilized to support these processes. Once the net photosynthesis of the new shoots exceeds the carbon demands of growth and maintenance, root storage is replenished (B to C).

Most studies on the role of carbohydrates during resprouting of woody plants have been in Mediterranean-type (Bowen & Pate 1993; Canadell & Lopez-Soria 1998) and forestry systems (Landhäusser & Lieffers 2002; Latt *et al.* 2000) with few studies in savannas (Hoffmann *et al.* 2000; Miyanishi & Kellman 1986). One year after a single fire, root starch concentrations of resprouting *Miconia albicans* and *Cildemia sericea,* two neotropical savanna shrubs, were 30% depleted compared to unburnt plants (Miyanishi & Kellman 1986). One year after 5 successive annual burns, the root starch concentration of the plants was 50% depleted. Two years after the burn, the root starch concentration of the shrubs had recovered to pre-fire levels. Root starch concentrations of *Stirlingia latifolia,* a Mediterranean-type shrub growing in south west Australia, decreased by 50 to 75% during the first 2 to 5 months after topkill, but recovered to pre-fire levels within 1.5 to 2 years (Bowen & Pate 1993). Neither the neotropical savanna shrubs (Miyanishi & Kellman 1986) nor the Mediterranean shrub (Bowen & Pate 1993) resprouted if they were topkilled when their root reserves were less than 10 percent of pre-disturbance levels. Saplings of *Populus tremuloides,* an important forestry species, rapidly recovered leaf area and replenished root starch reserves within 1 year if they were topkilled when root reserves were high (Landhäusser & Lieffers 2002). However, if the saplings were topkilled when reserves were low, leaf area recovery was slow, root reserves remained depleted after one year, and root growth suffered.

Although some carbohydrate reserves must be essential to initiate resprouting, carbohydrate reserves do not necessarily limit continued resprouting vigour (Chapin *et al.* 1990) as a number of studies have shown that resprouting plants still have high levels of reserves after resprouting from a single topkill event (Hoffmann *et al.* 2000, Wildy & Pate 2002). Resprouting by pot grown *Kielmeyera coriacea* seedlings, a common cerrado tree species, was not limited by root carbohydrate reserves, which remained greater than 36% after clipping and did not drop further when most of the shoot regrowth occurred (Hoffmann *et al.* 2000). Reserves would only become limiting for resprouting if topkill events happened very frequently or were timed such that the amount of reserves used during recovery, either for growth or for respiration, could not be replenished between the disturbances (Miyanishi & Kellman 1986; Bowen & Pate 1993).

Bush encroachment, Climate Change and Prescribed fires

Over the last century bush encroachment has become an increasing threat to rangelands and savannas in Africa (Hoffman & O'Connor 1999; Roques *et al.* 2001; Skowno *et al.* 2001), in Australia (Fensham *et al.* 2005), in North America (Asner *et al.* 2003; Archer 1995), and in South America (Silva *et al.* 2001). Bush encroachment is used here to describe both the expansion and densification of woody plant populations into grasslands and in savannas (Hoffman *et al.* 1999). Bush encroachment is undesirable in rangelands and savannas as it can decrease herbaceous production (Lett & Knapp 2005; Stuart-Hill & Tainton 1989; Watson & MacDonald 1983) and thus lower the carrying capacity of these systems (Ward 1962; Hoffman *et al.* 1999). Bush encroachment can also have a negative effect on biodiversity as typically only a few species are responsible for the encroachment but many species, particularly herbaceous species, are suppressed (Hobbs & Mooney 1986; Roques *et al.* 2001).

Recently bush encroachment has become even more of a concern as it is hypothesized that that increased atmospheric CO_2 concentrations will benefit woody plants more than herbaceous plants (Bond & Midgley 2000; Hoffmann *et al.* 2000; Polley *et al.* 2002). Woody plants rely on perennial carbon rich structures, i.e. stems and roots, thus improved photosynthetic capacity and allocation to wood in one year, due to increased atmospheric CO_2 concentration, has a cumulative benefit over the lifetime of the plant. Grassy plants, on the other hand, do not rely on large perennial structures, and most of their investment in carbon is naturally shed from one year to the next. Thus unlike woody plants, any benefit of improved photosynthesis and growth of grasses in one year is not carried over into following years (Bond & Midgley 2000). Accelerated growth and recovery of reserves between topkill events, due to increasing CO_2 concentrations, could have a significant effect on plants that depend on the life-history strategy shown in Fig. 1.1. It is hypothesized that by accumulating larger reserves that support rapid recovery after topkill plants could escape the fire-trap more easily and thus attain reproductive maturity sooner (Bond & Midgley 2000; Bond & Archibald 2003).

There is a long history of using pre-scribed fires to manage bush-encroachment in savannas (Bond & Archibald 2003; Brooks & MacDonald 1983; Govender *et al.* 2006; Trollope 1984; Ward 1962). However many savannas tree species are particularly fire resistant and the injudicious use of fire, particularly frequent low intensity fires, can promote invasion by fire resistant species, rather than prevent bush encroachment (Ward 1962; Whateley & Porter 1983; Trollope 1984). In the context of increasing atmospheric CO_2 concentrations resprouting woody plants might become even more fire-resistant (Bond & Midgley 2000). In lieu of long-term fire experiments, the resprouting physiology of different species could be used to inform management decisions on the appropriate fire regime that would favour desirable species and restrain the expansion of undesirable species (Miyanishi & Kellman 1986; Bowen & Pate 1993).

Thesis structure

This study investigated the resprouting characteristics of *Acacia karroo* that allow it to survive frequent and repeated topkill. *A. karroo* is a diverse species but the Hluhluwe form, found in KwaZulu–Natal, South Africa, is noted as being particularly fire tolerant (Bond *et al.* 2001) and provides a model type of the life history strategy described by Bond and van Wilgen (1996) and Gignoux *et al.* 1997 (Fig. 1.1) . It is also noted as a key species responsible for bush encroachment in South African savannas (Hoffman & O'Connor 1999; O'Connor 1995). Although *A. karroo* is not undesirable at low population densities as it is palatable and favoured by browsers it can form dense thickets that exclude fire and thus accelerate invasion by forest and true thicket species (Ward 1962; Whateley & Wills 1996). I hypothesized that the resprouting vigour of *A. karroo* saplings caught in the fire-trap would depend on their root starch reserves. Furthermore, I wanted to know whether frequent topkill would progressively lower the carbohydrate reserves of *A. karroo* saplings and thus their resprouting vigour. To test the hypothesis I broke up my thesis into a series of questions that addressed the various aspects of the carbon budget of resprouting plants shown in Fig. 1.2. These questions formed the basis of the first four data chapters and are as follows:

Chapter 3 – Root starch depletion and recovery after topkill

- Do root starch concentrations of *A. karroo* decline during resprouting?
- How quickly are reserves replenished after topkill?
- Does the resprouting vigour of *A. karroo* decrease if plants are topkilled annually?

Chapter 4 – The use of stored reserves for shoot growth during resprouting

- Do coppicing shoots of topkilled *A. karroo* saplings rely on root reserves for growth?
- For how long after topkill are roots independent from concurrent photosynthesis?

Chapter 5 – Leaf area renewal and photosynthesis of topkilled plants

- How do concurrent photosynthesis and root storage compare as sources of carbon during resprouting?
- Can new shoot growth of resprouting plants be accounted for by concurrent photosynthesis?

Chapter 6 – The effect of shading and defoliation on root starch replenishment

- Can shading or defoliation prevent the replenishment of root reserves after topkill?

In the last data chapter, Chapter 7, the storage patterns of other savanna trees and shrubs are investigated. The hypothesis tested in this chapter was whether fire-tolerant woody species preferentially allocate reserves carbohydrates to their roots, which are protected from fire, rather than their stems, which are vulnerable to fire damage.

Chapters 1, 2, and 8 make up the introduction, the description of the study area and species, and the conclusion, respectively.

Note to readers

Each of the data chapters were written as independent and potentially publishable units, thus, there is some repetition across the chapters, particularly in the introduction and methods sections.

Chapter 2 : Study area and species

Study area

The study was conducted in the Hluhluwe–iMfolozi Park (HiP), KwaZulu–Natal, South Africa (28°00′–28°26′ S; 31°43′–32°09′ E). HiP is a 900 km^2 fenced nature reserve that lies in the foothills of the first escarpment on the western side of the Zululand coastal plain (Whateley & Porter 1983). It is comprised of three smaller reserves, the Hluhluwe Reserve, the Corridor, and the iMfolozi reserve. Generally altitude, rainfall, and fire frequency decrease from Hluhluwe, in the north east, to iMfolozi, in the south west (Balfour & Howison 2001) (Fig. 2.1). The terrain of the park is varied, comprising both valleys and hills with an altitudinal range of 60 to 450 m above sea level (Whateley & Porter 1983). The soils are generally eutrophic and are largely derived from sandstone, shale, and dolerite intrusions (Balfour & Howison, 2001). The mean annual rainfall ranges from 990mm in the high altitude regions in the north west of the park to less than 635 mm in the low-lying southern regions (Balfour & Howison 2001). The majority of the rain falls during summer thunderstorms between October and March (Balfour & Howison 2001; Whateley & Porter 1983). Between 2002 and 2006, the mean daily minimum temperature recorded the Hluhluwe reserve in the coldest months of the year, June and July, was 12°C. Over the same time, the mean of the daily maximum temperature recorded in the warmest months of the year, January and February, was 31°C (Zululand Grass Project Weather Data) (Fig. 2.2).

The vegetation of the park is predominantly fine-leaved Acacia savanna (Whateley & Porter 1983), with a fairly open tree canopy and a continuous grass layer (Fig. 2.3). The Hluhluwe Reserve is characterized by *Acacia nilotica* woodlands, *Acacia karroo – Dichrostachys cinerea* thicket, and *Euclea divinorum* woodlands (Whateley & Porter 1983). There are also *Celtis africana* forest communities in Hluhluwe, especially at higher altitudes in the north-western corner of the reserve (Whateley & Porter 1983). The Corridor is characterized by *A. nilotica* woodlands and *A. karroo – D. cinerea* thicket (Whateley & Porter 1983). The iMfolozi Reserve is characterized by *Acacia nigrescens* woodlands, *Acacia tortilis* woodlands, and *Spirostachys*

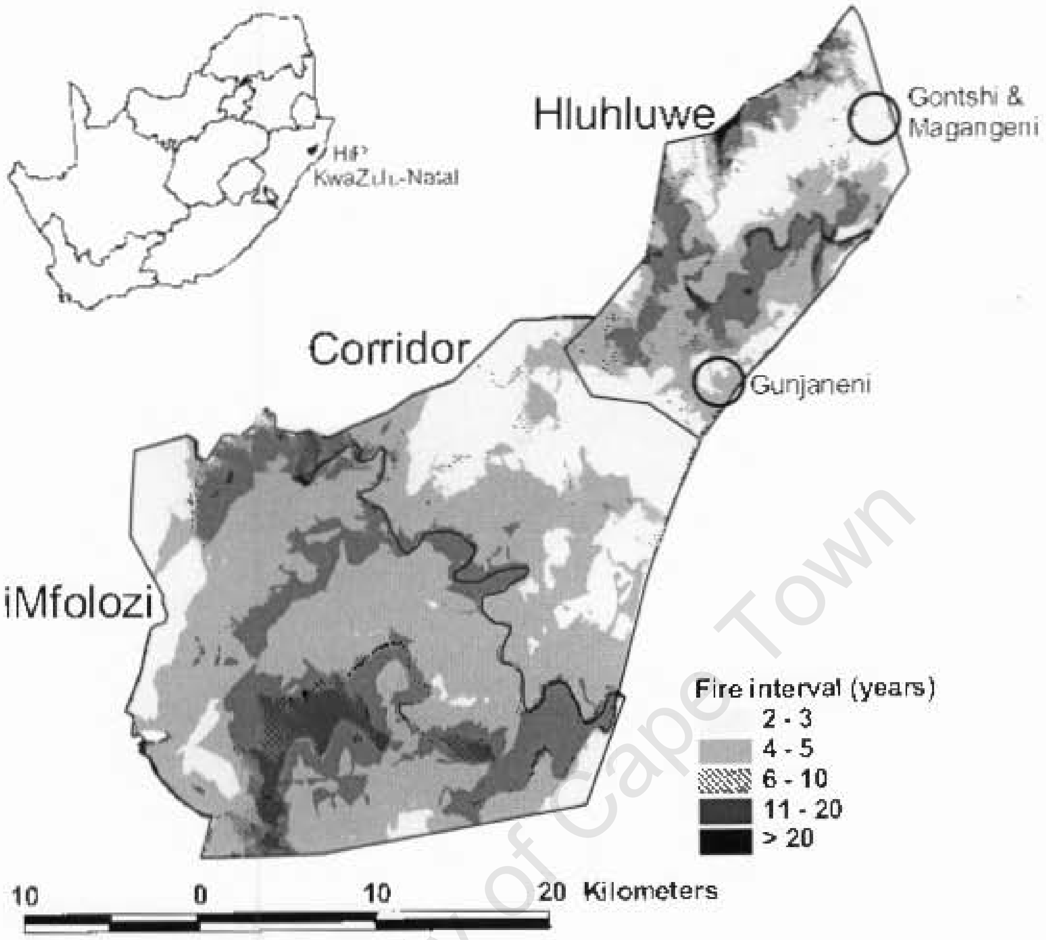

Fig. 2.1. A map of Hluhluwe-iMfolozi Park (HiP) showing the location of the study sites, Gunjaneni, Gontshi, and Magangeni and the mean fire intervals recorded in the park between 1956 and 1998. The inset shows the position of HiP in KwaZulu-Natal, South Africa.

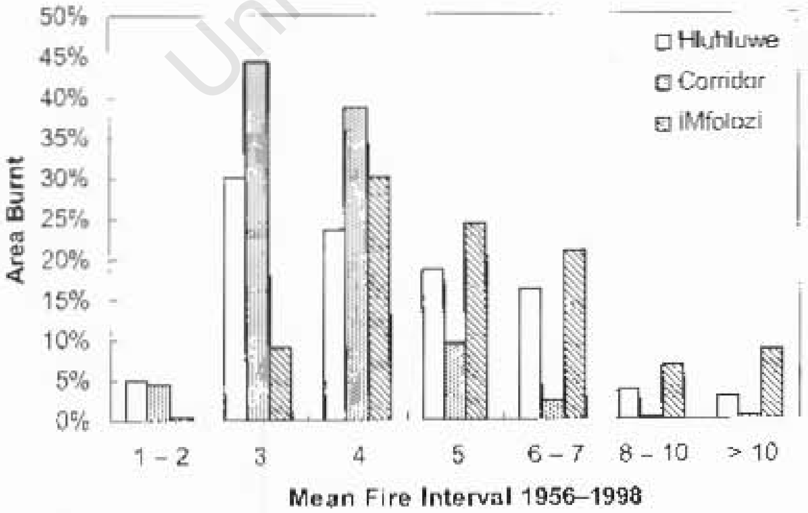

Fig. 2.2. The area burnt under different mean fire intervals for the three reserves between 1956 and 1998 (Ezemvelo KZN Wildlife, HiP fire records).

africanus woodlands (Whateley & Porter 1983). The *A. nilotica* woodlands (Fig. 2.3) and *A. karroo – D. cinerea* communities (Fig. 2.4 & Fig. 2.6) that dominate the Hluhluwe and Corridor reserves are both associated with tall dense grass swards that burn relatively frequently and intensely (Whateley & Porter 1983). These grass swards are predominantly made up of tall tufted perennial species from the Andropogoneae tribe such as *Themeda triandra* and *Cymbopogon excavatus* (Whateley & Wills 1996).

The warm wet summers promote the development of dense grass swards that cure and become highly flammable in the dry winter months. Between 1956 and 1996, the mean fire return interval for the entire park was 4 years, whereas the median was 1.3 years (Balfour & Howison 2001). Approximately 90% of the fires recorded over this period burnt between June and September, i.e. in the dry season (Balfour & Howison 2001). Fire frequency across the park is not uniform, and is highest in the Corridor and lowest in the iMfolozi Reserve (Fig. 2.1 & Fig. 2.2). Between 1956 and 1998, *ca.* 50% of the Corridor, 35% of the Hluhluwe Reserve, and only 15% of the iMfolozi Reserve burnt on average at least once every 3 years (Fig. 2.2) (Ezemvelo KZN Wildlife, HiP fire records). The distribution of the most frequently burnt areas in the Hluhluwe and Corridor Reserves (Fig. 2.1) corresponds largely to the distribution of *A. karroo – D. cinerea* communities (Whateley & Porter 1983).

As in most savannas, the fires in HiP are generally grass-fuelled surface fires (Fig. 2.5). The fires rarely cause complete plant mortality, as many of the local tree species are capable of resprouting (MacDonald 1980; Ward 1962). Nevertheless, there is a long history of using prescribed fires in an attempt to manage tree and shrub density in HiP (Brooks & MacDonald 1983; Ward 1962). One particular management goal has been the prevention of further encroachment of woody plants into the more open types of savanna within the reserve (Balfour & Howison 2001; Brooks & MacDonald 1983; MacDonald 1980). Similar to the global trend of bush encroachment over the last century, woody plants have been expanding their range in the reserve (Brooks & MacDonald 1983; Skowno *et al.* 1999; Watson & MacDonald 1983; Whateley & Wills 1996). Between 1937 and 1975, total woody cover in the north-eastern corner of the Hluhluwe Reserve increased by between 50 to 100 percent

(Watson & MacDonald 1983). *A. nilotica* woodlands have been especially vulnerable to encroachment (Whateley & Wills 1996), and over the last 50 years have transformed into broadleaved Euclea thickets (Skowno *et al.* 1999) and *A. karroo* savanna (Bond *et al.* 2001).

The park has a large complement of mammal herbivores (Brooks & MacDonald 1983). The large mammalian grazers in the bark include white rhino, African buffalo, wildebeest, zebra, and warthog. The large mammalian browsers in the park include black rhino, giraffe, and kudu. The large animals that consume both grass and browse, i.e. mixed-feeders, include elephant, impala, and nyala. Generally browsing intensity is highest in the winter months when the mixed feeders (impala and nyala) and even some of the grazers include more browse in their diets than at other times of the year (Botha & Stock 2005).

Fig. 2.3. An *A. nilotica* woodland in the southern end of the Hluhluwe Reserve. Note the continuous grass layer and the discontinuous tree layer characteristic of savannas. This photograph was taken in July 2005 in the middle of the dry season.

Fig. 2.4. *A. karroo* saplings growing in a tall dense grass sward of *Themeda triandra* and *Eragrostis curvula*. This photograph was taken in July 2005. The site had last burnt in September 2004.

Fig. 2.5. A fire sweeping across the landscape in September 2005. As in most savannas, the fire is largely fuelled by grass and runs along the ground. The tree in the centre of photograph on the right is an *A. nilotica* adult.

Fig. 2.6. Topkilled A. karroo saplings in an *A. karroo* *D. cinerea* thicket in September 2005 approximately 3 weeks after a burn. The unburnt *A. karroo* saplings on the left of the firebreak have already put out new leaves for the start of growing season.

Study Species

Acacia karroo Hayne is widely distributed in South Africa, and is found from the south-western tip of the country all the way into the north eastern corner (Brain 1989). The species is genetically diverse and can be divided into at least three types, a Karoo type, an Eastern Cape type, and a KwaZulu–Natal type (Brain 1989). Because of the genetic and phenotypic diversity within the *A. karroo* complex, the various forms of *A. karroo* are sometimes referred to as separate species. Thus, the Hluhluwe form of *A. karroo* is sometimes referred to as *A. natalitia* E. Mey. (Coates Palgrave 2002), however, in this thesis the original nomenclature is used. As the names imply, the three types have fairly distinct geographic boundaries but do overlap along these boundaries (Brain 1989). The Karoo type is found predominantly in the Karoo, in the south west of South Africa, but its range extends north as a tongue to the west of the Drakensburg Mountain Range (Brain 1989). The Eastern Cape type is found mainly in the Eastern Cape, but its range also extends into the north east of the country as a strip on the western border of the Drakensburg range (Brain 1989). The Natal type is found in a strip that is wedged between the eastern border of the Drakensburg and the KwaZulu–Natal coast, but its range also extends into the north east of the country but along the eastern border of the Drakensburg (Brain 1989).

The Natal race, and particularly the form found in HiP, is phenotypically different from the Karroo and Eastern Cape races. The Hluhluwe form is slender and sparsely branched, whereas the Karroo and Eastern Cape forms are highly branched and can be still be multi-stemmed as adults (Archibald & Bond 2003). The Hluhluwe form also has much smaller thorns compared to the Eastern Cape and Karroo varieties. In terms of structural defences, the Hluhluwe form is poorly defended against browsers, compared to the other *A. karroo* varieties, but rather seems better adapted to withstand frequent fire (Archibald & Bond 2003). As a juvenile, the Hluhluwe form of *A. karroo* is particularly abundant in tall-fire prone grasslands but rare on grazing lawns that are associated with intense herbivory (Bond *et al.* 2001). *A. karroo* seedlings are relatively tolerant of shade compared to other African acacias and are capable of establishing and surviving within a dense grass sward for at least one year (O'Connor 1995). The slender architecture of saplings of the Hluhluwe form of *A. karroo* is also an advantage in fire-prone habitats as it facilitates rapid vertical growth out of the zone of potential fire damage (Archibald & Bond 2003).

The Hluhluwe form of *A. karroo* is a model species of the life history strategy described by Bond and van Wilgen (1996) and Gignoux *et al.* (1997), whereby juvenile savanna trees survive frequent topkill events, by repeatedly sprouting from their fire-protected rootstock. Plants with this life-history can persist for decades in stunted non-reproductive stage, surviving fire after fire, until they eventually manage to grow tall enough to escape fire damage and mature into reproductive adults (Trollope 1984). In one of the few post-fire censuses conducted in HiP, mortality of *A. karroo* was only 2.3% one year after the fire (MacDonald 1980). The mortality rate of unburnt *A. karroo* plants over the same period was 1.6% (MacDonald 1980).

After topkill, new shoots emerge from the root crown of the plant (Fig. 2.7) within weeks after the fire. Typically, numerous coppice shoots will emerge after the fire (Fig. 2.8) and these coppice shoots can persist from a few months to years before one of the shoots achieves apical dominance and the plant reverts into a single stemmed stage (Fig. 2.9). Once a stem has been topkilled, it quickly disintegrates, leaving no aboveground evidence of the true age of the plant. However, after repeated topkill, saplings develop swollen taproots with the scars of previous stems that succumbed to fire (Fig. 2.10). During the transition stage between a sapling and a young adult, the Hluhluwe form of *A. karroo* is slender and sparsely branched and is rarely reproductive. Once the adults have grown out of the fire-trap they become more branched (Fig. 2.11) and reproductively mature (Fig. 2.12).

Fig. 2.7. New shoots emerging from the rootstock of a topkilled *A. karroo* sapling.

Fig. 2.8 The coppice growth of topkilled *A. karroo* saplings. This photograph was taken approximately 5 weeks after a fire late in August 2005.

Fig. 2.9. A 1.5 year old stem of an *A. karroo* sapling. The previous stem is clearly visible in the alongside the new stem. This photograph was taken in April 2006 after the site had burnt in September 2004.

Fig. 2.10. The swollen taproots of *A. karroo* saplings showing evidence of previous topkilled stems. Note the discrepancy in the basal area of the stems and the roots, particularly in the photograph on the right. The photograph on the left shows the remains of at least two dead stems that were presumably topkilled in previous fires, as indicated by the circles.

Fig. 2.11. *A. karroo* adults. Note the slender sparsely branched architecture of the young adult on the left. The adult shown on the right was in flower at the time of the photograph in January.

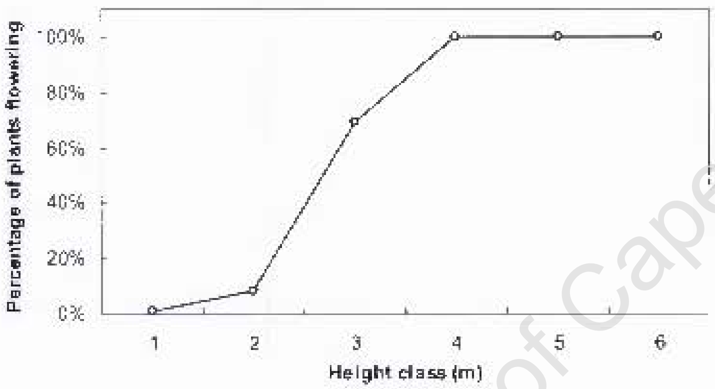

Fig. 2.12. The percentage of *A. karroo* plants flowering in different height classes. The data was collected in January 2006 in seven 50 m × 2 m transects in the southern end of the Hluhluwe Reserve (n = 179).

A. karroo is generally noted as one of the key species responsible for bush encroachment in KwaZulu-Natal (Bond *et al.* 2001; Hoffman & O'Connor 1999) and in South Africa as a whole (Hoffman *et al.* 1999; O'Connor 1995). In the Weenen/Muden area of KwaZulu–Natal, which is to the south of and slightly more inland than HiP, there was an increase in woody cover from a mean of 21% in 1955 to 59% in 1998 (Hoffman & O'Connor 1999). This change was largely driven by increasing dominance of *A. karroo* at higher altitudes and *A. tortilis* at lower altitudes. Since the 1960s, there has been a trend in HiP of *A. karroo* expansion into what were previously more open types of savanna (Bond *et al.* 2001). In the 1980s in *A. nilotica* woodlands, *A. nilotica* juveniles were nearly three times as abundant as *A. karroo* juveniles (Whateley & Porter 1983). By the late 1990s, this pattern had reversed and *A. karroo* juveniles were more than three time as abundant as *A. nilotica* juveniles (Bond *et al.* 2001).

Chapter 3 : The depletion of root reserves during resprouting of *Acacia karroo* after topkill

Introduction

Fire intervals in mesic savannas can be as short as 1 to 3 years (Bond & Midgley 2000; Frost & Robertson 1987; Hoffmann & Solbrig 2003). The surface fires typical of savannas often kill the aboveground parts of short trees and shrubs, but plant mortality after the fires is usually low (Glitzenstein *et al.* 1995; Trollope 1984; Williams *et al.* 1999) because many of the plants resprout from the surviving belowground tissues (Bond & van Wilgen 1996; Frost & Robertson 1987; Gignoux *et al.* 1997; Trollope 1984). The death of aboveground parts that leave a surviving root is referred to as topkill. After topkill, the carbon requirements for growth of new tissues and the maintenance of surviving tissues, mostly roots, must be met from root carbon stores until current assimilation of the coppicing shoots is sufficient to meet the growth and respiration demands of the plant (Landhäusser & Lieffers 2002; Langley *et al.* 2002).

Plant storage can be defined as the accumulation of resources that can be mobilized to support biosynthesis and other plant functions (Chapin *et al.* 1990) when the rate of utilization exceeds that of current production (Kozlowski 1992). The storage of carbohydrates and subsequent mobilization of these reserves to support particularly respiration but also growth has been clearly demonstrated for deciduous tree-crops, such as apple (Loescher *et al.* 1990; Tromp 1983). These plants accumulate carbon stores over autumn, and mobilize these reserves during bud break and spring growth (Loescher *et al.* 1990). Post-burn resprouting of woody plants is in some ways analogous to the spring growth of deciduous trees; both experience rapid shoot elongation at a time when the total leaf area of the plant is small. Like the spring growth of deciduous trees, resprouting of trees and shrubs is associated with depletion of carbohydrate reserves, particularly starch (Bowen & Pate 1993, Canadell & Lopez-Soria 1998, McPherson & Williams 1998; Miyanishi & Kellman 1986; Sakai & Sakai 1998). One year after a single fire, root starch concentrations of resprouting *Miconia* *albicans* and *Cildemia sericea,* two neotropical savanna shrubs, were 30% depleted compared to unburnt plants, and 50% depleted 1 year after 5 successive annual burns (Miyanishi & Kellman 1986). Two years after a burn the reserves had returned to pre-fire levels (Miyanishi & Kellman 1986). Root starch concentrations of *Stirlingia latifolia,* a Mediterranean-type shrub growing in South West Australia, where fires occur every 5 to 7 years, decreased by 50 to 75% during the first 2 to 5 months after topkill, but recovered to pre-fire levels within 1.5 to 2 years after the fire (Bowen & Pate 1993). Although plants do not resprout if root starch reserves reach low enough levels (i.e. less than 10% of the pre-disturbance level) (Bowen & Pate 1993; Canadell & Lopez-Soria 1998; Miyanishi & Kellman 1986), only a fire regime of annual fires would progressively lower the starch reserves of *M. albicans* and *C. sericea* (Miyanishi & Kellman 1986), and *S. latifolia* (Bowen & Pate 1993). Thus, in terms of storage and resprouting potential, these resprouting shrubs are well adapted to survive frequent fires.

To survive repeated disturbances, such as fire, a plant must reoccupy its original "site", and thus secure access to resources, at least between disturbance events but also before other competing plants can exclude it (Bellingham & Sparrow 2000; Midgley 1996). In savannas, where the grass sward can recover fast enough to allow annual fires (D'Antonio & Vitousek 1992), the height growth of the new coppicing shoots will have to match that of the grass, if the shoots are to escape considerable shading at a time when high photosynthetic rates might be critical for the replenishment of used carbohydrate reserves. The short interval between fires in mesic savannas means that resprouting plants need to both recover the aboveground parts necessary to support the plant, outgrow competitors, and replenish the reserves used during recovery within 1 to 3 years.

Despite the importance of fire as a management tool in the savanna parks of South Africa (Bond & Archibald 2003; Govender *et al.* 2006) and the prevalence of resprouting woody species in these parks (Bond & Archibald 2003; Luoga *et al.* 2004; Trollope 1984), there has been little work on processes that affect the resprouting capacity of native savanna trees and shrubs (but see Maze 2001). *Acacia karroo* Hayne has been singled out as one of the most important grassland invaders in South Africa (O'Connor 1995), and also specifically in the Hluhluwe–iMfolozi Park (HiP), KwaZulu–Natal (Bond *et al.* 2001; Skowno *et al.* 1999), where it seems particularly well adapted to tolerate frequent fire (Archibald & Bond 2003; Bond *et al.* 2001; Walters *et al.* 2004). For example, the aboveground stem architecture of the slender form of *A. karroo,* found in the Hluhluwe–iMfolozi Park, facilitates rapid height gain into size classes that are invulnerable to the ground fires typical of savannas (Archibald & Bond 2003). This study investigated (1) whether root starch was depleted during resprouting of *A. karroo*; (2) whether the reserves, if used, were replenished between topkill events; and (3) whether the resprouting vigour of *A. karroo* was reduced if topkilled annually.

Methods

Study site

Fieldwork for the study was conducted at three sites within the north-eastern half of the Hluhluwe–iMfolozi Park (HiP), KwaZulu–Natal, South Africa. The park is a 900 km^2 fenced game reserve. The vegetation of the park is predominantly fine-leaved Acacia savanna (Balfour & Howison 2001). The park has a mean annual rainfall ranging from 990mm in the north-western hills to less than 635 mm in the low lying southern regions (Balfour & Howison 2001). Most of the rainfall falls during summer thunderstorms (Balfour & Howison 2001). Between 1956 and 1996 the mean fire return interval for the park was 3.8 years, and the median fire return interval was 1.3 years. Over the same period, approximately 90% of the fires burnt between June and September (i.e. in the dry season) (Balfour & Howison 2001).

The coordinates, elevation and mean fire return interval of the three sites are shown in Table 3.1. All three sites burnt in October 2004 and August 2005, however, at each of the sites there were adjacent patches of burnt and unburnt vegetation which allowed the comparison of burnt and unburnt plants. The rainfall at three weather stations within 5 kilometres of the Gunjaneni site was 637±99 mm (mean ± SE) between July 2004 and June 2005, and 507±70 mm between July 2005 and June 2006 (Zululand Tree Project, University of Cape Town). The soils at the sites were dark brown, fine textured, slightly acidic (pH *ca.* 5.5) soils, derived from dolerite parent material (HiP Research Centre, soils map). The tree layer at the sites was characterised by

Sclerocarya birrea and *Acacia nilotica* adults, and *A. karroo* and, to a lesser extent, *A. nilotica* juveniles. The shrub layer consisted of *Dichrostachys cinerea*, and less often *Eulcea divinorum*, as well as localized patches of *A. caffra* and *Maytenus senegalensis*. The grass community at the sites were more diverse than the tree and shrub layer, but overall, *Themeda triandra*, *Panicum maximum*, *P. deustum* and *Eragrostis curvula* were the most common of the grass species present.

Table 3.1: Site descriptions

Site	Co-ordinates	Elevation (m)	Mean (±SE) Fire return interval 1956–2005
Gunjaneni	28.202°S, 32.042°E	140	3.8 ± 0.4
Gonthsi	28.056°S, 32.129°E	200	2.3 ± 0.1
Magangeni	28.064°S, 32.125°E	150	2.3 ± 0.1

Root starch depletion and replenishment

The pattern of root starch depletion and replenishment during aboveground recovery was tracked by harvesting the taproot of *A. karroo* saplings that burnt in August 2005. The plants were single stemmed and between 1.2 to 2.0 m tall before the fire. The pre-fire height of the plants was determined by measuring the height of the skeleton of the old stem, or if the dead stem had collapsed then the diameter of the old stem was used to gauge the pre-fire size using a regression equation (Height (cm) = 47×stem diameter (cm) + 52, R^2=0.85, n=78, $p<0.001$). In July 2005, five unburnt plants were harvested from the Gontshi site. In December 2005, six burnt and six unburnt plants were harvested from the Gunjaneni site. In February 2006 and in August 2006, seven burnt and seven unburnt plants were harvested at each of the three sites. For each plant harvested, 30 cm of the root, measured from the lowest belowground stem scar, was excavated. Immediately after returning from the field, the roots were dried at 70°C for 5 days and then weighed. The roots were milled using a Wiley mill (Arthur H. Thomas Co., Philadelphia, USA) with a 1 mm mesh and analysed for root starch content, as described later. To test whether there was a significant difference between the root starch concentration of the burnt and unburnt plants collected in February and August 2006 a factorial ANOVA was used with the site, the harvest date, and the treatment (burnt or unburnt) included as categorical predictors, and root starch concentration included as the response variable. A Post-hoc Tukey test was used to determine the significant differences between the individual means, which are reported at $p < 0.05$. The 2005 data was not included in the statistical analysis as samples were not collected at all three sites during the 2005 harvests.

Above and below ground recovery after successive topkill events

The new shoot growth of saplings and adults that were topkilled twice, once, or not at all was recorded every 3 to 4 months over *ca.* 2 years. The saplings were single stemmed and between 60 to 150 cm tall before topkill, whereas the adults were single stemmed and between 220 to 420 cm tall before topkill. After a fire in October 2004, 20 unburnt adults, 20 burnt and topkilled adults, 20 unburnt saplings, and 20 burnt and topkilled saplings were tagged, and the pre-fire height and stem diameter of the burnt plants was measured using skeletons of the dead stems. In July 2005, half of the plants in each group were clipped at ground level to simulate a second topkill event. An unplanned fire in August 2005 burnt all the initially unburnt plants, so unfortunately by the end of the sampling there were no plants that had not been burnt at least once during the investigation. A summary of the various treatments is shown in Table 3.2. Aboveground growth measurements were taken approximately every 3 months between November 2004 and July 2006. For resprouting plants the height of the new regrowth; the stem diameter of each shoot, taken *ca.* 5 cm from the base of the shoot; and the length of each shoot were recorded. For unburnt plants the height of the plants and the stem diameter, taken from *ca.* 5cm from the base of the plant, were recorded. Root samples were taken from all plants in February 2005 and July 2006, using root cores of sap wood (diameter 5 mm, 10 mm long) taken from the taproot at *ca.* 5 cm below the soil. The root cores were dried at 70°C for 48 h, milled using a Wiley mill (Arthur H. Thomas Co.) with a 1mm mesh, and the ground root material was analysed for starch content.

To test the hypothesis that root starch would be depleted after a single topkill event, a factorial ANOVA was used with the size class and the treatment (burnt or unburnt) as the categorical predictors and the February 2005 root starch concentrations as the response variables. To test the hypothesis that root starch would be further depleted by annual topkill events, a factorial ANOVA was used with the size class and the treatment (Table 3.2) included as categorical predictors and the July 2006 root starch concentrations included as the response variables. To test whether repeated topkill would have an effect on the growth rate of the trees, repeated measures ANOVAs were performed with size class and treatment (Table 3.2) used as categorical predictors, and the measurements of plant height were used as the response variables. Significant differences between individual means were determined using Post-hoc Tukey tests, and are reported at $p < 0.05$.

Table 3.2: Summary of treatments included in the analysis of the above and below ground recovery rates of saplings and adults after repeated topkill.

Treatment	Description
Topkilled in 2004 only	These plants were topkilled in 2004 during the October burn and were protected from topkill in 2005.
Topkilled in 2004 and 2005	These plant were topkilled in 2004 during the October burn and again in July 2005 by clipping the plants at the base of their stems.
Topkilled in 2005 only	These plants were not burnt in 2004 but were topkilled in 2005. The adults were topkilled by clipping the plants at their base of their stems in July 2005, whereas the saplings were topkilled by the August 2005 fire.

Starch analysis

Staining of thin sections of root tissue with a 2% iodine/potassium iodide solution showed that starch was spread uniformly, both longitudinally and transversely, in the sapwood of the taproots of saplings (Fig. 3.1). The tissue sections (20μm) were cut on a base-sledge microtome (Reichert-Jung, Austria) and washed in alcohol to remove tannins and pigments before they were stained with the iodine solution (Johansen 1940).

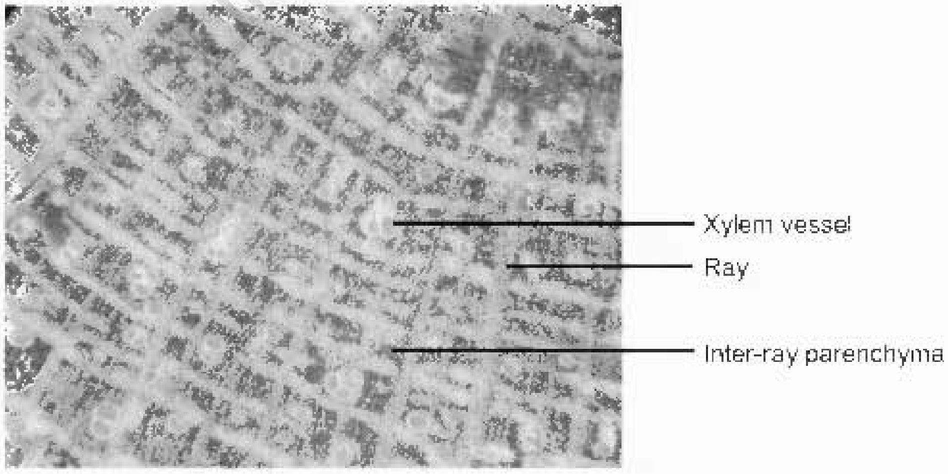

Fig. 3.1: A cross-section of root tissue from the taproot of a sapling stained with iodine. Starch was present in the inter-ray parenchyma cells, as indicated by their dark colouring.

Starch in sub-samples (50 mg) of dried, ground root material was hydrolysed to glucose using the Amyloglucosidase–alpha-amylase method described by McCleary *et al.* (1997). The glucose concentration of the resulting solution was determined colorimetrically using the phenol–sulphuric method described by Dubois *et al.* (1956). Prior to the starch determination, soluble sugars were removed from the sub-sample by washing the sub-sample in hot (80°C) 80% ethanol (v/v) for 5 minutes, centrifuging the solution at 760 × *g* for 10 minutes, and discarding the supernatant. This process was repeated three times. Starch was hydrolyzed to glucose by incubating the residue remaining after the sugar extraction for 5 minutes at 100°C with 300 U thermo-stable alpha-amylase (from *Bacillus lichen*, Sigma-Aldrich (Pty) Ltd, Castle Hill, New South Wales, Australia); and then incubating the solution for 1 h at 55°C after adding 20 U purified amyloglucosidase (from *Aspergillus niger*, Sigma-Aldrich). The hydrolysate was centrifuged at 760 × *g* for 10 minutes, and the supernatant was used in the colorimetric procedure. For the colorimetric procedure, 200 μl of 5% phenol was added to 200 μl of sample, and 1 ml of concentrated sulphuric acid was added to the mixture. The colour was allowed to develop for at least 15 minutes before the absorbance was read at 490 nm with a microplate spectrophotometer (PowerWave XS, BioTek, Winooski, Vermont, USA).

Results

Starch depletion and replenishment

The root starch concentrations of both the burnt plants and the unburnt plants followed a seasonal cycle of depletion during spring and summer and replenishment through autumn and into mid winter (Fig. 3.2). In December 2005, 4 months after the August 2005 fire, the mean root starch concentration of the unburnt plants had decreased from the July value of 0.34±0.04 g g^{-1} to 0.27±0.02 g g^{-1}, while the mean concentration of root starch of topkilled plants had decreased even further to 0.17±0.02 g g^{-1}. Between December 2005 and February 2006, the root starch concentration of the topkilled plants and the unburnt plants decreased at a similar rate. By the end of the summer the mean root starch concentration of unburnt plants was *ca.* 50% of the winter maximum, and the mean starch concentration of the topkilled plants was *ca.* 25% of the winter maximum. A factorial ANOVA of the February 2006 and August 2006 data showed that both the season ($F_{1,75}$=360, p<0.0001) and the treatment, i.e. topkilled or unburnt, ($F_{1,75}$=24, p=0.0001) significantly affected the mean root starch concentration of the plants. But there was also a significant interaction between the two factors (season × treatment, $F_{1,75}$=8.4, p=0.005), probably because the root starch concentration of the burnt plants was replenished more rapidly than that of the unburnt plants. By August 2006, the mean root starch concentration of the topkilled plants was only slightly less than that of the unburnt plants.

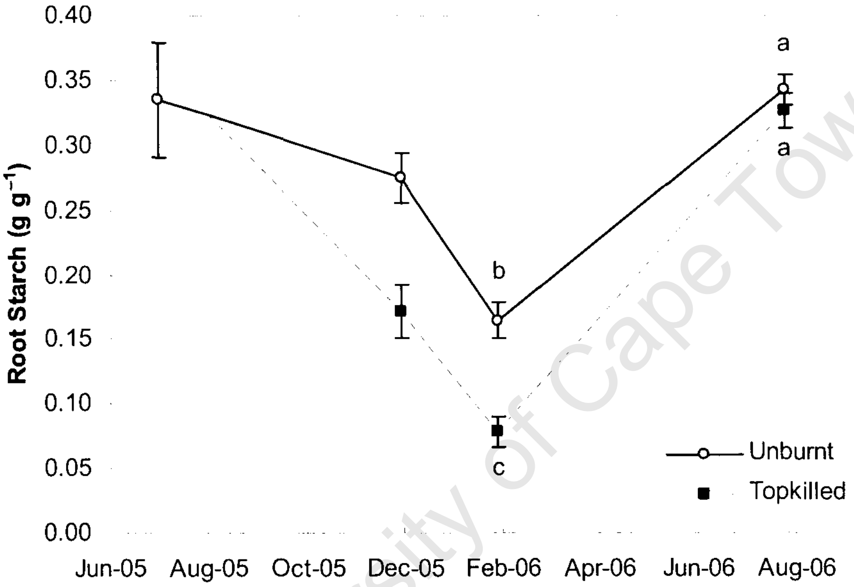

Fig. 3.2: The mean (±SE) root starch concentrations of unburnt and burnt plants collected between July 2005 and August 2006. The topkilled plants had been burnt in August 2005. The symbols indicate the means of the data from all three sites. The letters indicate significant differences according to a Post-hoc Tukey test (p < 0.05). (Only the data from the last two harvests were included in the statistical analysis as before this, samples were not collected at all three sites.)

Above and below ground recovery after successive topkill events

The root starch concentration of adults was generally lower than that of saplings in February 2005 ($F_{1,68}$=22.8, p<0.0001) (Fig. 3.3a) and in July 2006 ($F_{1,61}$=20.2, p<0.0001) (Fig. 3.3b). Root starch concentration of both the adults and the saplings followed a similar pattern of depletion and replenishment during the first growing season after topkill and up to the end of the study in July 2006. In February 2005, there was a significant treatment, i.e. topkill, effect ($F_{1,68}$=7.1, p=0.01), and the mean starch concentration of topkilled plants was less than that of unburnt plants for both the adults and the saplings (Fig. 3.3a). By July 2006, the treatment effect was no longer significant. The mean root starch concentration of the saplings topkilled in 2004 and 2005 was only slightly less than that of saplings topkilled in 2004 only and even higher than the mean of saplings topkilled in 2005 only (Fig. 3.3b). The root starch concentration of adults that had been topkilled twice was less than that of adults that had been topkilled once only in 2004 and once only in 2005 but the differences were not statistically significant.

Although the root starch concentration of saplings and adults differed significantly in July 2006, the adults had much larger roots, and thus the total root starch content (root starch concentration × root mass) of the saplings and adults in July 2006 was not significantly different ($F_{1,58}=1.2$, $p=0.3$). The mean root basal diameter, at *ca.* 5 cm below the surface, of the adults in July 2006 was 6.7±0.3 cm compared to 4.0±0.2 cm for the saplings (Two-tailed t-test, $t_{62}=8.4$, $p<0.0001$).

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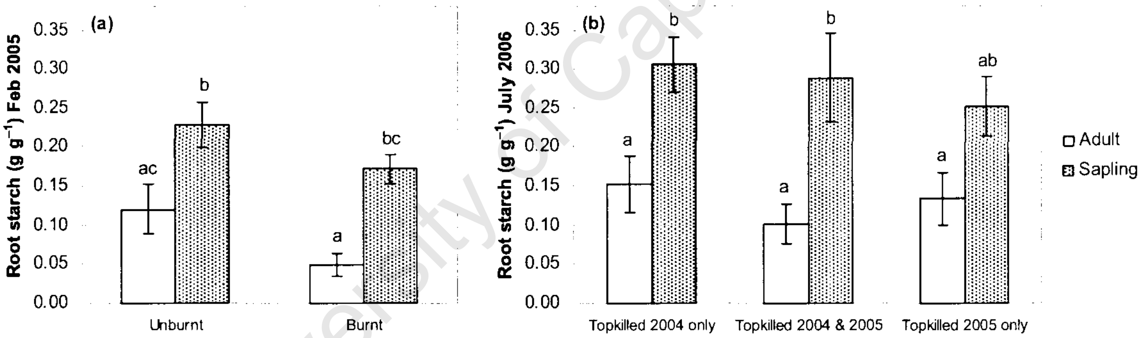

Fig. 3.3: (a) The mean (±SE) root starch concentration of unburnt adults (clear bars) and saplings (shaded bars) compared to burnt adults and saplings in February 2005 after the fire in October 2004; (b) and the mean (±se) root starch concentration of plants in July 2006 after half of the plants had been topkilled for a second time in July 2005 (see Table 3.2). The letters indicate significant differences ($p < 0.05$).

Aboveground recovery

Regardless of the size class or the treatment, on average all resprouting plants grew taller than 50cm within one season after topkill. The resprouting saplings generally grew taller than the resprouting adults in the corresponding treatment, although the differences were not statistically significant (Fig. 3.4). Both the adults and saplings that were topkilled in 2004 and again in 2005 grew to heights after the second topkill that were similar to the heights they grew to after the first topkill (Fig. 3.4b).

Furthermore, the plants topkilled twice grew slightly taller, although not significantly so, after the second topkill than the plants, in the corresponding size class, that were topkilled once only in 2005 (Fig. 3.4b). The saplings that had two growing seasons to recover from topkill were on average slightly taller than they had been before the fire in October 2004, but this difference was not statistically significant.

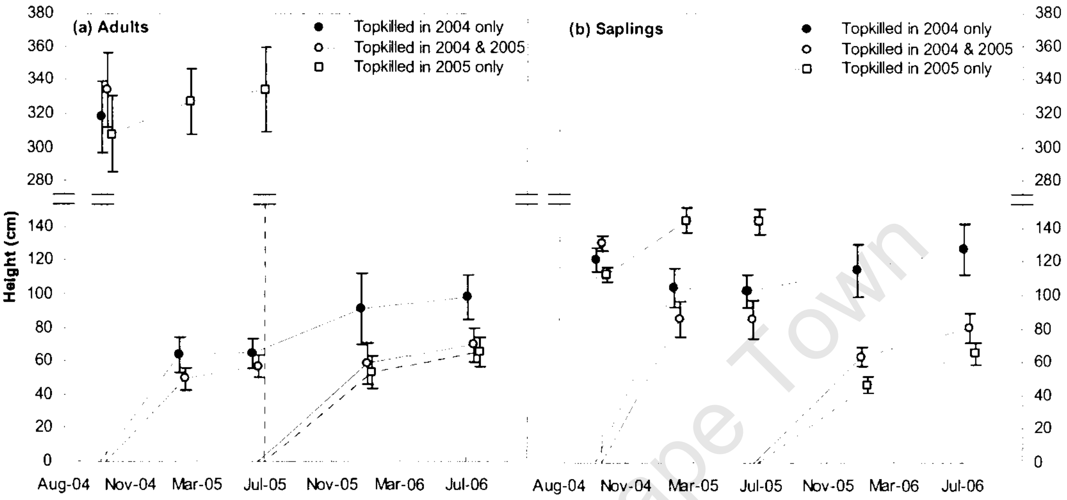

Fig. 3.4: The mean (±SE) height of (a) adults and (b) saplings were topkilled in 2004 only, in 2004 and 2005, and in 2005 only (see Table 3.2).

The pattern of shoot growth during resprouting was characterised by initial rapid shoot elongation and a more gradual thickening of the shoots (Fig. 3.5); this pattern was especially pronounced after the 2004 fire. Generally shoot elongation of the recently resprouting plants ceased at the end of the summer in February, whereas shoot thickening continued through autumn and into mid winter in July. Between February 2005 and July 2005 the mean length of the largest shoot of resprouting adults and saplings hardly changed for the adults (Fig. 3.5a) and the saplings (Fig. 3.5b), whereas the mean shoot diameter of adults (Fig. 3.5c) increased nearly 2-fold, and the mean shoot diameter of the saplings (Fig. 3.5d) increased by approximately 1.5-fold. The rapid growth after topkill was largely confined to the first post-fire growing season, thus the plants that were topkilled in 2004 only failed to repeat the growth spurt of the first growing season after topkill in the second growing season (Fig. 3.5).

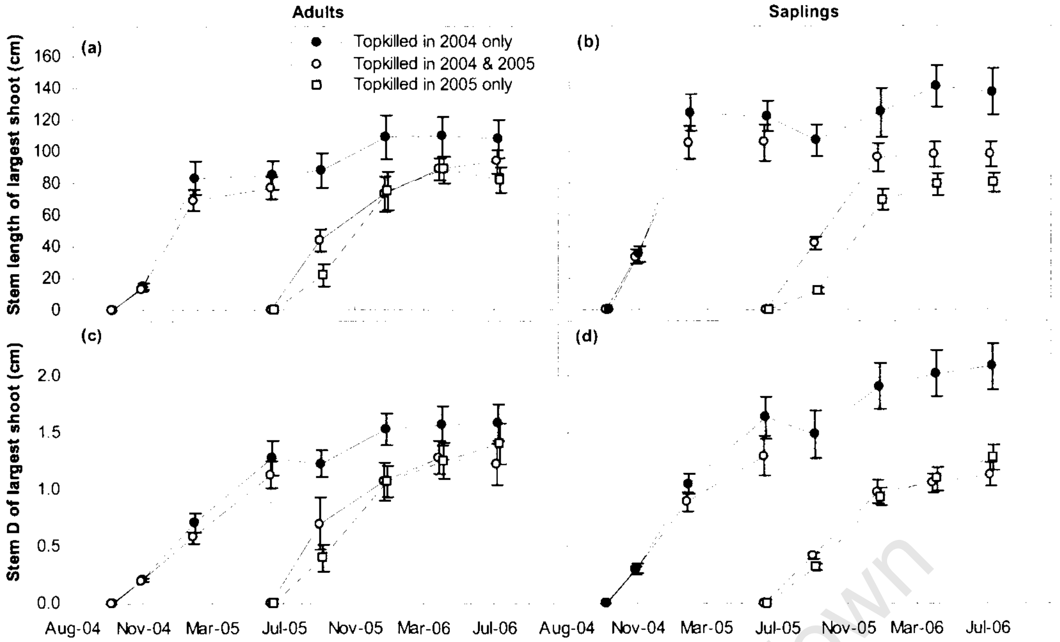

Fig. 3.5: Shoot growth of the resprouting adults and saplings was characterised by rapid initial shoot elongation (a and b) and more gradual shoot thickening (c and d). The symbols represent means (±SE).

Discussion

In February 2006, 6 months after topkill, the mean root starch concentration of the resprouting *A. karroo* plants was approximately one-half that of the unburnt plants and only a quarter the pre-fire level (Fig. 3.2). Thus it is seems that for *A. karroo,* as has been reported for other resprouting woody plants, starch reserves are mobilized to supplement either growth (Bowen & Pate 1993) or the respiratory demands of the surviving root (Landhäusser & Lieffers 2002; Langley *et al.* 2002) during the first growing season after topkill. The rapid replenishment of starch reserves within 1 year after topkill (Fig. 3.2) was unexpected, and suggests that even annual fires would not decrease the resprouting vigour of *A. karroo* saplings.

The ability of resprouting *A. karroo* saplings to replenish spent starch reserves within a year after topkill was confirmed by the observation that the root starch concentration of saplings that had been topkilled twice over two consecutive years matched that of saplings that been topkilled once and had had two years to recover (Fig. 3.3). The height growth of plants topkilled twice in two consecutive winters also matched the height growth of plants topkilled only once over the two years (Fig. 3.4). This is not to say that the fire regime cannot affect the size class structure of a population of *A.*

karroo. The adults, that were on average 3m tall before the October 2004 fire, were not able to grow much taller than 1m if they were topkilled in two consecutive years (Fig. 3.4). However, this study does indicates that under a regime of annual burns, plants that have grown to a height of 100 cm at least once are maintained within the 50 to 100 cm size class, regardless of their pre-fire size. Although the plants do not necessarily recover their pre-fire heights, or reoccupy their original "site" (see Bellingham & Sparrow 2000), they do manage to grow taller than the grass layer, and thus enjoy high light levels for remainder of growing season and from the start of the next growing season.

The depletion of root starch reserves during resprouting took place under a background cycle of seasonal starch depletion and replenishment. During spring and into summer the starch concentration of roots of unburnt *A. karroo* saplings decreased by 50%, and then increased to a mid-winter maximum during autumn and early winter. Depletion of reserves during spring, and particularly during canopy construction, is typical of plants growing in strongly seasonal climates (Chapin *et al.* 1990; Kozlowski 1992; Loescher *et al.* 1990). The depletion is often greatest when the area of the canopy of the plants is at a maximum (Cruz & Moreno 2001; Newell *et al.* 2002; Tissue & Wright 1995; Wyka 1999). Reserves of deciduous plants such as neotropical forest understory shrubs (Tissue & Wright 1995) and the alpine herb *Oxytropis sericea* (Wyka 1999) are depleted and replenished within the growing season. The reserves of semideciduous neotropical forest tree species (Newell *et al.* 2002) and *Erica australis*, an evergreen Mediterranean shrub (Cruz & Moreno 2001), are depleted over the growing season and replenished during the dry season, as is the case for *A. karroo*. The ability of resprouting *A. karroo* saplings to replenish spent reserves within only 1 year after topkill might be due the rapid canopy expansion of the resprouting plants in spring, and the maintenance of the canopy into the dry season when conditions for growth deteriorate. The shoot growth of both the resprouting adults and the saplings was characterised by initial rapid expansion up until February (Fig. 3.5), during which time current assimilation was supplemented by root starch reserves (Fig. 3.2). After February, which marks the end of the summer and the wet season proper, shoot elongation ceased, whereas root starch replenishment commenced and continued into winter, as did shoot thickening. Thus, by February the current assimilation of the new shoots was sufficient to support both shoot thickening and root starch replenishment. The allocation switch in late summer from shoot growth to belowground sinks has also been reported for neotropical (Franco 1998; Hoffmann 2002) and Australian (Prior *et al.* 1997; 2004) savanna trees. The photosynthetic rates of these neotropical (Franco 1998) and Australian (Prior *et al.* 1997; 2004) savanna trees remained relatively high into the dry season whereas their shoot growth was confined predominantly to the wet season. Although this might represent an active strategy of allocation to storage as an adaptation to the high risk of topkill in savannas (Franco 1998; Hoffmann 2002; Prior *et al.* 1997; 2004), it could also be a forced physiological response to declining water and nutrient availability (Chapin *et al.* 1990; Knox & Clarke 2005; Mooney & Chu 1974). Generally, growth of plants is more sensitive to stress than photosynthesis is (Lambers *et al.* 1998) and in savannas nutrient and water availability is generally greatest in the wet summer months (Bernhard-Reversat 1982; Scholes & Walker 1993). Nevertheless, the ability of resprouting plants to accumulate large reserves when conditions are not suitable for growth can be seen as an adaptation in itself (Knox & Clarke 2005).

Most fires in the Hluhluwe–iMfolozi Park (Balfour & Howison 2001), and in South African savannas generally (Scholes & Archer 1997; Trollope 1984), occur in the dry season. The root starch reserves of *A. karroo* were also greatest during the dry season. This is in contrast to the Mediterranean-type resprouting shrub, *Erica australis*, in Spain where starch reserves are low in summer when the risk of fire is high (Cruz & Moreno 2001). Topkill in the middle of the growing season, when starch root reserves were low, resulted in poor resprouting vigour of *Adenostoma fasciculatum*, a chaparral shrub, (Jones & Laude 1960) and saplings of north American hardwood trees (Kays & Canham 1991) and also poor root retention and root starch replenishment of *Populus tremuloides* saplings (Landhäusser & Lieffers 2002). A long term fire experiment in a loblolly pine plantation in Florida, USA, found that mid-growing season burns caused more mortality of understory hardwoods and shrubs than dormant season burns at the same frequency (Robbins & Myers 1992). Like *A. karroo*, these hardwood species are topkilled by fire but are able to resprout (Robbins & Myers 1992). In mesic Australian savannas, fires in the early dry season, when the plants are still physiologically active, retard the height growth of juvenile trees, whereas fires in the late dry season, when the plants are dormant, do not (Prior *et al.* 2006). Thus the timing of topkill can affect the resprouting response of plants. When plants are burnt when they are actively growing and starch reserves are low they resprout less vigorously than if they are topkilled in the dormant season (Drewa 2003; Jones & Laude 1960). However, the timing of the fire also affects the intensity of fires, and therefore the probability of topkill (Higgins *et al.* 2000; Trollope 1984). Topkill is more likely in the dormant season (Drewa 2003; Govender *et al.* 2006; Higgins *et al.* 2000; Trollope 1984), when the grass sward is cured and highly flammable, than in the growing season, when the grass sward is green and less flammable.

Conclusion

A. karroo is well adapted to a regime of frequent dormant season fires. In late winter root starch reserves of *A. karroo* are maximal and can supplement rapid post-burn shoot growth which then has the remainder of the growing season and the start of the dry season to replenish spent reserves. Annual dormant season fires can change the size class structure of an *A. karroo* population so that even old plants are kept shorter than 100 cm, but the plants do manage to grow taller than the grass layer even under repeated annual burns. The rapid replenishment of belowground reserves facilitates the persistence of the smaller size classes that are vulnerable to topkill. This study only included two consecutive topkill events. It is possible that an extended period of annual topkill, or a regime of growing season fires, would cause a more substantial decrease in the resprouting vigour of plants and the eventual eradication of encroaching plants.

Chapter 4 : The transport and use of old and new assimilates during resprouting of *Acacia karroo*

Introduction

In mesic savannas fire-intervals can be as short as 1 to 3 years (Bond & Midgley 2000; Frost & Robertson 1987; Hoffmann & Solbrig 2003), and although above ground stems of shrubs and small trees are often killed by the fires, many of the plants survive (Glitzenstein *et al.* 1995; Trollope 1984; Williams *et al.* 1999) by resprouting from the root collar (Bond & van Wilgen 1996; Frost & Robertson 1987; Gignoux *et al.* 1997; Trollope 1984). The saplings of *Acacia karroo*, which is a widespread species in South African savannas (Brain 1989), can survive at least eight successive annual fires by coppicing from the root after each burn (Trollope 1984). During resprouting of woody savanna shrubs (Miyanishi & Kellman 1986) and Mediterranean-type heath shrubs (Bowen & Pate 1993; Canadell & Lopez-Soria 1998) belowground non-structural carbohydrate (NSC) reserves drop by 30 to 75 percent. If NSC reserves reach low enough levels these plants do not resprout (Bowen & Pate 1993, Canadell & Lopez-Soria 1998; Miyanishi & Kellman 1986). Thus, root NSC reserves are thought to be an important indicator of the ability of a plant to resprout (Jones & Laude 1960).

The role of NSC reserves during bud break and shoot elongation has been extensively studied for deciduous tree-crops (Hansen 1967; Kandiah 1979; Loescher *et al.* 1990; Tromp 1983) and commercially grown pines (Glerum & Balatinecz 1980; Gordon & Larson 1970; Lippu 1998) using ^{14}C labelling techniques. The advantage of ^{14}C studies is that they can distinguish between current assimilates and stored labelled assimilates. These studies have shown that the majority of reserve NSCs mobilized during bud break and shoot elongation are used in respiration (Glerum & Balatinecz 1980; Gordon & Larson 1970, Hansen 1967; Kandiah 1979; Lippu 1998; Tromp 1983). For deciduous tree-crops, only 13 to 25 percent of the mobilized NSCs are used as building material for the new shoots (Hansen 1967; Hansen & Grauslund 1973; Kandiah 1979); for evergreen pines, the contribution can be as low as 4 percent

(Glerum & Balatinecz 1980). Although NSC reserves contribute quantitatively little to the regrowth of the grass *Themeda triandra,* the reserves indirectly promote growth by funding an initial growth spurt of photosynthetic tissue that then becomes the major source of carbohydrates (Danckwerts 1993). Similarly, although reserve carbohydrates of the biennial *Arctium tomentosum* contributed less than 1 percent to above ground growth in its second year, the reserves primed the growth of the first leaves so that in the second year leaf development was completed in 7 weeks compared to 20 weeks in the first year (Heilmeier *et al.* 1986). ^{14}C studies on tree-crops have also shown that during shoot development basipetal transport is severely restricted for up to 4 weeks after bud break (Lockwood & Sparks 1978b). During this period the photosynthates from the first leaves are directed towards the younger leaves and the developing shoot tip (Kandiah 1978, Lockwood & Sparks 1978a, Lockwood & Sparks 1978b). Thus, during shoot elongation root maintenance respiration would be supplied from root reserves (Loescher *et al.* 1990). But root activity of trees is not necessarily limited to maintenance in the absence of basipetal translocation of photosynthates (Jordan & Habib 1996) as root reserves can contribute to new root growth (Langley *et al.* 2002; Lockwood & Sparks 1978a; Lockwood & Sparks 1978b; Srivastava *et al.* 1986).

Despite the prevalence of resprouting in savanna ecosystems, few studies have considered the physiological aspects that control resprouting in savannas (but see Hoffmann *et al.* 2000; Miyanishi & Kellman 1986). ^{14}C labelling provides a useful technique to track the stored assimilates that are mobilized during resprouting. Unlike the spring flushing of deciduous trees and biennials, and the resprouting of grasses (which are all systems that have been investigated using ^{14}C labelling), resprouting woody plants need to rebuild their stem system as well as their canopy and thus might be more reliant on reserves as building materials because of the proportionally high initial investment in non-photosynthetic tissues. Furthermore, spring flushing trees often draw down both stem and root reserves (Lockwood & Sparks 1978a), but resprouting trees have lost their above ground stores so their root reserves would presumably be used both for shoot production (albeit mostly for respiration costs), root maintenance, root growth, and nutrient acquisition. This study used ^{14}C pulse labelling to determine (1) whether coppicing shoots of topkilled *A. karroo* saplings rely on root reserves for growth, and (2) for how long after aboveground death roots are independent from new photoassimilates.

Methods

Experimental design

Forty-eight *A. karroo* Hayne plants were grown from seeds collected in Hluhlwe–iMfolozi Park (HiP), KwaZulu-Natal, South Africa. Seed dormancy was broken by lightly sandpapering the seeds, to damage the hard testa (seed coat), and then soaking the seeds in hot water overnight or until they had imbibed. The seeds germinated within a few days after imbibing and were planted on the 15th November, 2004, into 75 L bins filled with acid washed sand. Each bin was divided into four partitions with one plant per partition. The plants were grown in the University of Cape Town's glasshouse, Rondebosch, South Africa. Each plant was supplied with 400ml 2 mM N_2 Long Ashton nutrient solution three times a week. The plants were supplemented with water as needed, which varied from 4 minutes of spray twice a day during the height of summer to 4 minutes once a week in winter. In September 2005, all the plants were clipped back to a height of 1m, after which they sprouted new shoots from the tip of the remaining stem. By the time the ^{14}C labelling experiments were conducted in February 2006, the mean basal area of the plants was greater than 1cm, and the plants were between 0.8 and 1.2 m tall. Two ^{14}C labelling experiments were conducted. For the first experiment (Fig. 4.1a), shoots of intact plants were labelled with ^{14}C, and then clipped at the base, i.e. topkilled, 2 days later and allowed to resprout. For the second experiment (Fig. 4.1b), plants were first clipped at the base and allowed to coppice before they were labelled with ^{14}C.

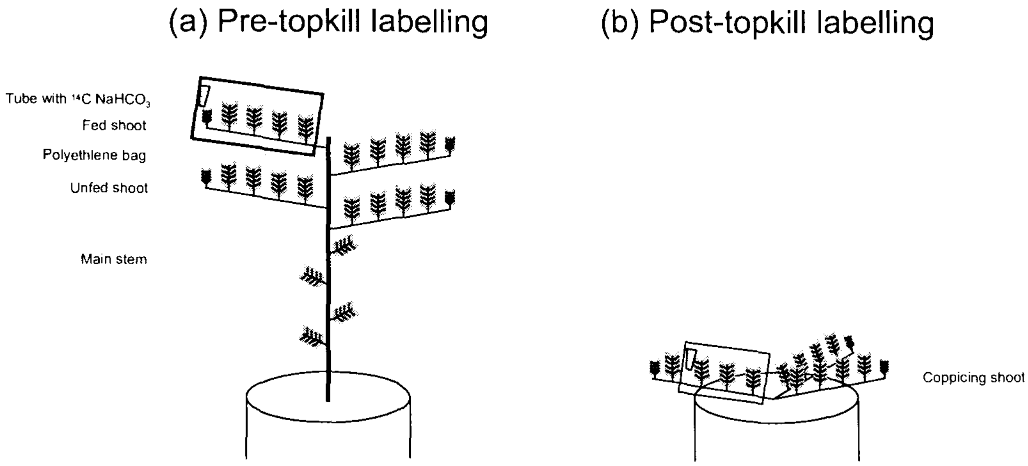

Fig. 4.1: The setup of the two ^{14}C labelling experiments. (a) For the pre-topkill labelling experiment, 20 plants were labelled with ^{14}C, clipped at the base 2 days later and harvested over 6 weeks. (b) For the post-topkill labelling experiment, 16 plants were clipped at the base of the stem and then labelled with ^{14}C after the new shoot had emerged.

^{14}C labelling

Pre-topkill labelling

On the 22 February, 2006, 20 plants were pulse labelled for 2 h with $^{14}CO_2$. Entire shoots (40-60cm long) were sealed into custom made polyethylene bags (volume *ca.* 4L) along with 2ml tubes that held 120 µl of $NaH^{14}CO_3$ with a total activity of 40 µCi or 1480 Bq (Amersham Biosciences, Buckinghamshire, UK) (Fig. 4.1a). One dominant shoot was used per plant. After the bags were sealed onto the shoot they were covered with black plastic bags to limit photosynthesis, and thus prevent the drawdown of the CO_2 concentration in the bag, until $^{14}CO_2$ was released from the $NaH^{14}CO_3$ solution by adding 1 ml of 10% lactic acid (v/v). Before the black bags were removed, the clear polyethylene bags were gently palpated to facilitate mixing of the released $^{14}CO_2$ throughout the volume of the bag. After a cold chase period of 2 days to allow for the translocation of ^{14}C photosynthates into the root system, all the plants were clipped at the base of the stem. The stem and shoots were divided into the fed leaves, the fed stem, the unfed leaves, the unfed shoots, the stem leaves, and the stem. Over the next 6 weeks as the plants resprouted, five sets of four plants were harvested on the 24th February, 14th March, 23rd March, 30th March, and 7th April 2006. On the harvest date, the sand was washed off the roots after they had been carefully extracted from the sand. The plant material was separated into coarse roots (diameter > 3mm), fine roots (diameter < 3mm) and nodules, developing leaves, young shoot tips, fully expanded leaves, and mature shoot bases. The plant material was dried at 70ºC for 72 hours, weighed, and ground to a powder using a Wiley mill (Arthur H. Thomas Co., Philadelphia, USA) with a 1mm sieve-plate.

Post-topkill labelling

Sixteen plants were clipped at the base on the 16 February, 2006, and allowed to coppice and produce new leaves before they were labelled with $^{14}CO_2$. Plants were labelled as described earlier except that the bags were initially open on both sides so that they could be sealed below the apex of the fed shoot to exclude the young leaves from the bag (Fig. 4.1b). The bags were also smaller (*ca.* 1L) than the bags used for the pre-topkill–labelled plants as the shoots were generally much shorter. Sets of four plants were labelled at a time. Not all the plants resprouted, and therefore there were only three labelling events, instead of the planned four. Plants were labelled on the 21st March, 28th March, and 5th April, 2006. For each plant, the longest of the new shoots was labelled. In the first and last labelling events, one of the plants was damaged in each case, so the number of replicates for these events was reduced from four to three. Plants were harvested, dried, weighed, and milled as for the pre-topkill–labelled plants, except that in this case, the unfed expanding leaves and young shoot tips that were on the fed shoot were kept separate from the leaves and stems of the unfed shoots.

Starch extraction and analysis

Starch was extracted from subsamples (50 mg) of ground coarse root material following the Amyloglucosidase–alpha-amylase method described by McCleary *et al.* (1997). Prior to the starch assay, soluble sugars were removed by washing the sample in hot (80°C) 80% ethanol (v/v) for 5 minutes, centrifuging the solution for 10 minutes at 760 × g, and discarding the supernatant. The sugar extraction process was repeated three times. Starch was hydrolyzed to glucose by incubating the remaining residue for 5 minutes at 100°C with 300 U thermo-stable alpha-amylase (from *Bacillus lichen*, Sigma-Aldrich (Pty) Ltd, Castle Hill, New South Wales, Australia), and then incubating the solution for 1 h at 55°C after adding 20 U purified amyloglucosidase (from *Aspergillus niger*, Sigma-Aldrich). Once the starch hydrolysis procedure was complete, the solution was centrifuged for 10 minutes at 760 × g, after which 2 ml of the supernatant was set aside to be counted for ^{14}C activity, and the glucose concentration of the remaining supernatant was determined colorimetrically using the Phenol–Sulphuric method of Dubois *et al.* (1956). For the colorimetric procedure, 200 µl of 5% phenol was added to 200 µl of sample, and then 1 ml of concentrated sulphuric acid was added to the mixture. The colour was allowed to develop for at least 15 minutes before the absorbance was read at 490 nm with a microplate spectrophotometer (PowerWave XS, BioTek, Winooski, Vermont, USA).

^{14}C Counting

The ^{14}C of 50 mg sub-samples of ground plant material were released from the sub-samples and captured using a Sample Oxidiser (Packard Tricarb Model 306, Packard Instruments, Downers Grove, Illinois, USA). The Sample Oxidiser incinerates the sample, captures the released CO_2 in 8 ml of Carbo-sorb (Perkin Elmer, Waltham, Massachusetts, USA) and then dispenses the Carbo-sorb and 12 ml of Permafluor (Perkin Elmer) into a scintillation vial. The 2 ml of supernatant set aside for counting from the starch extraction procedure was added to 14 ml of Ready-Gel (Beckman-Coulter, Inc., Fullerton, California, USA) in a scintillation vial. The samples were counted using a Beckman LS 5000 TD liquid scintillation counter (Beckman Instruments, Fullerton, California, USA). Counts Per Minute (CPM) were converted to Disintegrations Per Minute (DPM) using a quenching curve based on clear Beckman ^{14}C quenching standards (Beckman Instruments), and DPM was converted to Becquerels (Bq), (DPM / 60). The amount of ^{14}C recovered in an organ or tissue was expressed as a proportion of the total ^{14}C label recovered in the plant including all harvested material, and as the specific activity (Bq g^{-1}) of the organ standardized for the amount of ^{14}C recovered in the source leaves of the particular plant compared to the mean amount of ^{14}C recovered in the source leaves of all the plants. These two measures were used because if an organ is particularly small the proportion of ^{14}C recovered in it can be small even though the organ is a strong sink relative to its size.

Results

Pre-topkill labelling

For the plants that were labelled with ^{14}C before they were topkilled, most of the recovered ^{14}C was found in the original above ground biomass (82±2%), and of this the majority was in the fed leaves (66±2%) and in the stem supporting these leaves (12±1%). As the coppicing shoots grew, the proportion of ^{14}C in the coppicing shoots increased linearly ($R^2=0.95$, $n=18$, $p<0.0001$) while the proportion of ^{14}C in the coarse roots ($R^2=0.62$, $n=18$, $p=0.0001$) and in the coarse-root starch ($R^2=0.40$, $n=18$, $p=0.005$) decreased exponentially (Fig. 4.2). The proportion of ^{14}C recovered in the fine roots initially decreased as the coppice shoots grew but then increased again, however, there was no statistically significant trend. The proportion of ^{14}C recovered in the coppice shoots was generally much lower than that recovered in the roots as a whole but similar to what was recovered in the root starch (Fig. 4.2b).

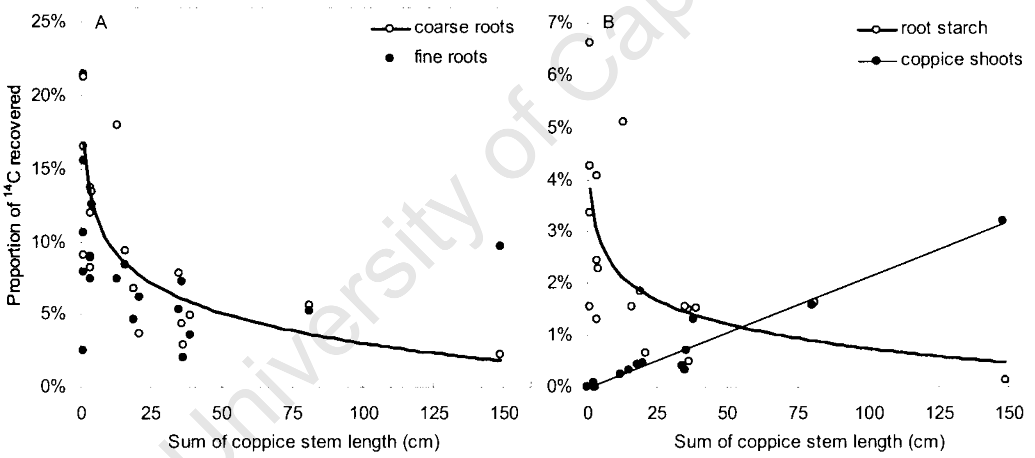

Fig. 4.2: The proportion of ^{14}C recovered in (a) the coarse and fine roots, and in (b) the coppice shoots and in the coarse root starch for the plants that were labelled with ^{14}C before they were topkilled

The standardized specific activity, specific activity hereafter, of the coppice shoot tissues was positively correlated to the specific activity of the coarse-root tissues (R^2= 0.77, n=11, p=0.0004) (Fig. 4.3); was generally greater than that of the coarse roots; and increased as a linear function of the specific activity of the coarse roots.

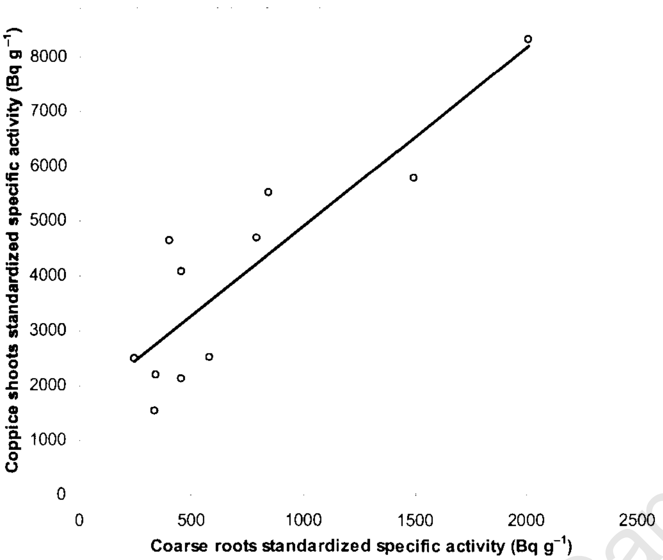

Fig. 4.3: The standardized specific activity of coppice shoot tissue of plants labelled before they were topkilled was generally greater than and positively correlated with the standardized specific activity of coarse roots (R^2=0.77, n=11, p=0.0004).

The specific activity of the coppice tissues decreased from *ca.* 7500 Bq g^{-1} to *ca.* 1000 Bq g^{-1} according to a power function as the new shoots developed. The R^2 values of the regression analyses between specific activity of coppice tissues and total shoot length were significant for all the tissues except the mature leaves (Fig. 4.4).

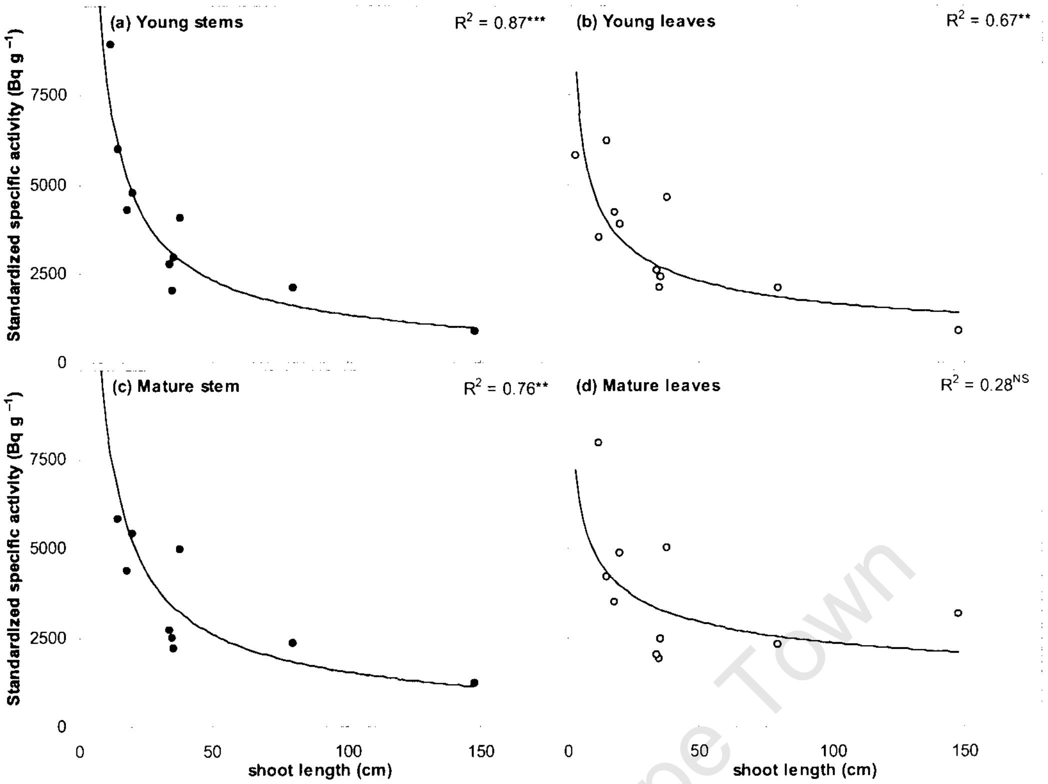

Fig. 4.4: The standardized specific activity of young and mature stems, and young and mature leaves of coppice shoots of plants that were labelled before they were topkilled. The standardized specific activity of all the coppice tissues decreased rapidly according to a power function as the new shoots grew (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Post-topkill labelling

Nearly all the label recovered in the tissues of the post-topkill–labelled plants was recovered in the aboveground parts (99.3±0.3%), of which most was found in the fed leaves (78±3%) and in the stem supporting the fed leaves (12±2%). As the shoots grew, the proportion of ^{14}C retained in the fed leaves decreased gradually, however the trend was not significant. The proportion of ^{14}C in the stem supporting the fed leaves increased linearly as the shoots grew ($R^2 = 0.40$, n = 10, p = 0.05) (Fig. 4.5a). The proportion of ^{14}C recovered in the fine roots also increased linearly as the shoot grew ($R^2 = 0.41$, n = 10, p = 0.05) but remained much lower than what was recovered in the aboveground tissues (Fig. 4.5b). Over the duration of the experiment, the mean proportion of ^{14}C recovered in the unfed young stem and leaves on the fed shoot was 6 % ± 2.5%, whereas it was only 0.3% ± 0.1% in the coarse roots and 0.4% ± 0.2% in the fine roots.

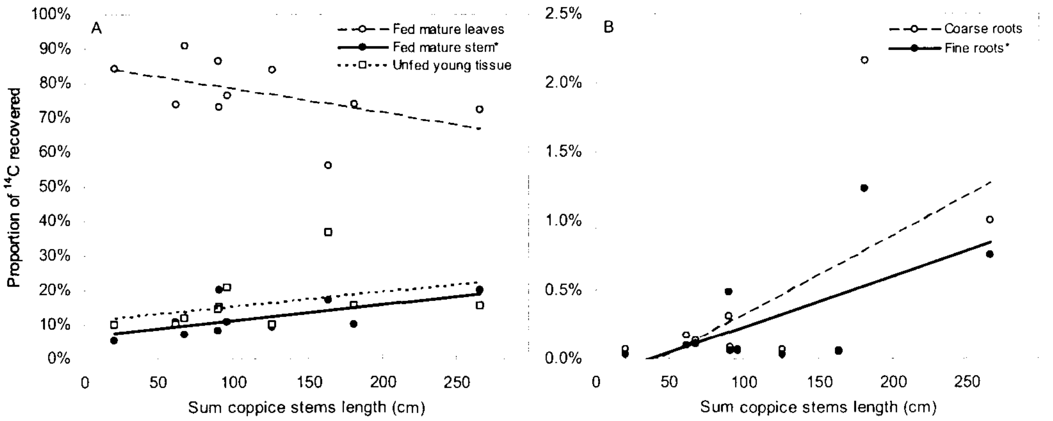

Fig. 4.5: The proportion of ^{14}C label recovered in (a) the fed leaves, the stem supporting the fed leaves, and the young unfed tissue on the fed shoot and in (b) the coarse roots and the fine roots (*$p < 0.05$).

Discussion

Most of the ^{14}C recovered in the plants that were labelled before they were topkilled was either in the fed leaves or in the stem tissue basipetal to the fed leaves. A longer chase period might have allowed more labelled photosynthates to reach the roots. Although labelled photosynthates can appear within the shoots and stem within a few hours after labelling of apple and pines, a longer period is needed for the assimilates to reach the roots (Hansen 1967), and it can take 3 to 6 days for assimilates to reach their final destination (Hansen 1967; Glerum & Balatinecz 1980).

After topkill, the labelled carbon in the coarse roots declined exponentially as the coppice shoots grew. The decline was not matched by an increase in the labelled C in the coppicing shoots or in the fine roots (Fig. 4.2), therefore, during this time nearly all of the ^{14}C mobilized in the coarse roots must have been respired. Most of the carbon respired during the first 5 days after clipping was not from starch, but probably from sucrose, as the proportion of labelled carbon recovered in the coarse roots decreased by *ca.* 13%, whereas the proportion of label in the coarse-root starch decreased by only *ca.* 3%. There was no significant change in the proportion of label recovered in the fine roots, so the slight decrease and subsequent increase of the proportion of ^{14}C recovered in the fine roots is difficult to interpret. But it is possible that the increase in the proportion of ^{14}C recovered in the fine roots was caused by the import of ^{14}C from the coarse roots, as basipetal transport from the new leaves on coppicing shoots to the roots was severely limited (Fig. 4.5b), and root reserves are known to contribute to new root growth of resprouting oaks (Langley *et al.* 2002) and spring flushing apple (Lockwood & Sparks 1978a; Lockwood & Sparks 1978b). Over the course of the pre-topkill labelling experiment, at most only *ca.* 19% of the ^{14}C label that was exported to the roots during the 2 day chase period was later incorporated into coppicing shoot biomass. This result agrees well with studies on tree-crops where only 13% to 25% of the non-structural carbohydrates (NSCs) mobilized during spring were allocated to new shoot biomass, while the rest was lost to respiration (Hansen 1967; Hansen & Grauslund 1973; Kandiah 1979).

Although the proportion of label recovered in the coppicing shoots of the plants that were labelled before they were topkilled was small, it steadily increased as the shoots grew (Fig. 4.2b), which indicates that stored assimilates continued to contribute to shoot growth even after the shoots were fairly well established. The strong and positive correlation between the standardized specific activity, specific activity hereafter, of the coarse roots and the coppicing shoots (Fig. 4.3) also indicates that the export of ^{14}C from the roots to the shoots continued even as the concentration of ^{14}C in the roots decreased. The specific activity of the new shoots was generally higher than that of the coarse roots. Kandiah (1979), working on apple, also found that the specific activity of new shoots was higher than that of the supporting branch. He proposed that either the ^{14}C label was not distributed evenly in the local reserves of the supporting branch and that a single component of high activity contributed to the new shoot growth, or that ^{14}C was imported from a more distant pool of reserves with a similar specific activity to the new shoot. This study supports the first explanation, i.e. uneven distribution of label, as in this system there was only one source of ^{14}C (the root NSCs), and its specific activity was less than that found in the new shoots. Thus it seems that recently labelled assimilated NSCs, that would have had a high specific activity, were mobilized, and partially used for shoot construction, in preference over older reserves on a "last in first out" basis (Jordan & Habib 1996).

Although the contribution from belowground reserves to new shoot biomass continued after the shoots were established, on a per gram basis the contribution rapidly declined (Fig. 4.4) as the shoots elongated. As the shoots elongated, the first leaves on the new shoots matured and started exporting assimilates particularly to the stem but also to the shoot tip (Fig. 4.5a). Thus, the first few leaves and shoot tips would have been more dependent on stored NSCs than the leaves and shoot that developed later, as the contribution from reserves became diluted by current photosynthates. A similar situation occurs during bud break of apple and pecan where the contribution of NSC reserves to the first 5 to 6 leaves can be as much as 50%–60% but is substantially less and quantitatively unimportant for the entire shoot (Hansen 1967; Kandiah 1979; Lockwood & Sparks 1978a). The contribution of stored reserves to rapid growth seems to be largely indirect by supplying the substrate and energy to re-establish leaf area rapidly when conditions become optimal for photosynthesis after defoliation or dormancy (Danckwerts 1993; Hansen 1967; Landhäusser & Lieffers 2002).

Throughout the experiment, basipetal transport from coppicing shoots was very limited. Most of the photosynthates of mature leaves of coppicing shoots were retained in the leaves themselves, exported to the supporting stem, and to a lesser extent transported acropetally (Fig. 4.5a). The proportion of ^{14}C recovered in roots 48 h after labelling of pre-topkill–labelled plants was ca. 25% (Fig. 4.2), whereas the proportion of ^{14}C recovered in roots 48 h after labelling of post-topkill–labelled plants was at most still less than 5% (Fig. 4.5b). Basipetal transport from new shoots to roots of apple and pecan during spring is also limited; photosynthates exported from the newly matured leaves during spring are directed to the younger expanding leaves and the elongating shoot tips (Hansen 1967; Kandiah 1979; Lockwood & Sparks 1978a; Lockwood & Sparks 1978b), and basipetal translocation only commences 4 weeks after bud break (Lockwood & Sparks 1978b). In this study, basipetal translocation from coppicing shoots had not commenced even after 7 weeks of coppicing. NSCs can support root functions in the absence of current photosynthates (Langley *et al.* 2002; Landhäusser & Lieffers 2002). For example, belowground reserves of resprouting scrub oaks in Florida can provide approximately a third of fine root and mycorrhizae carbohydrate requirements for up to 2.5 years after a fire (Langley *et al.* 2002). Root retention in woody plants is also dependent on belowground reserves (Landhäusser & Lieffers 2002). *Populus tremuloides* saplings that were topkilled in autumn, when belowground NSC reserves were high, suffered less root mortality compared to saplings that were topkilled in spring, when belowground reserves were low (Landhäusser & Lieffers 2002).

Conclusion

This study has shown that during resprouting of *A. karroo,* while the coppice shoots rapidly elongate, NSC reserves are used in a similar manner as they are for deciduous tree-crops during bud break; i.e., (1) the majority of mobilized reserves are used for tissue respiration in the very early stages of bud emergence and shoot elongation during resprouting, and only *ca.* 20% of mobilized reserves are incorporated into new shoot biomass; (2) the newly matured leaves quickly start to supplement the contribution from reserves to new shoot growth; and (3) basipetal translocation to the roots is limited during initial shoot elongation. The partitioning of new photosynthates of resprouting *A. karroo* differed from that of flushing tree-crops, in that basipetal translocation was delayed for longer, i.e. at least 7 weeks for *A. karroo* compared to 4 weeks for pecan (Lockwood & Sparks 1978b).

As with resprouting grasses (Danckwerts 1993) and 2nd year biennials (Heilmeier *et al.* 1986) the contribution of reserves to rapid shoot growth of *A. karroo* seems to be largely indirect by supplying the energy and substrate needed for the rapid recovery of photosynthetic tissue. The new leaves supply photosynthates almost exclusively to younger shoot tissues. This suggests that a key role of root reserves would be the maintenance of surviving root tissues during resprouting (Landhäusser & Lieffers 2002; Langley *et al.* 2002).

Chapter 5 : Rapid leaf area renewal of topkilled *Acacia karroo*—quantifying the role of concurrent photosynthesis and root reserves

Introduction

In fire prone environments, such as savannas (Bond & van Wilgen 1996; Gignoux *et al.* 1997; Hoffmann & Solbrig 2003), Mediterranean shrub ecosystems (Bowen & Pate 1993; Canadell & Lopez-Soria 1998) and Florida scrub (Langley *et al.* 2002; Olano *et al.* 2006), above ground death, or "topkill", due to fire is often followed by resprouting from below ground structures. Savannas and grasslands are the most fire prone biomes in the world (Mouillot & Field 2005); fire intervals in mesic savannas can be as short as 1 to 3 years (Bond & Midgley 2000; Frost & Robertson 1987; Hoffmann & Solbrig 2003). The potential for frequent fires in savannas results from the coincidence of a continuous highly flammable dormant grass sward and hot dry periods in the winter months (Mouillot & Field 2005). Fires in savannas are typically ground fires, thus once trees grow to a certain height they become less vulnerable to topkill (Trollope 1984). However, the short interval between fires in mesic savannas allow few opportunities for trees to grow to such a height. Many savanna trees survive for decades in a stunted often multi-stemmed form by resprouting time after time from belowground structures (Bond & van Wilgen 1996; Gignoux *et al.* 1997).

Although fires are recognised as one of the major disturbances in mesic savannas (Higgins *et al.* 2000; Sankaran *et al.* 2004; Scholes & Archer 1997) and despite the prevalence of resprouting trees and shrubs in savannas (Bond & Midgley 2000; Luoga *et al.* 2004), few studies have investigated the physiology of resprouting in savanna trees and shrubs (Hoffmann *et al.* 2000; Miyanishi & Kellman 1986). Belowground reserves of carbohydrates are often assumed to play a substantial role in the resprouting vigour of savanna trees (Bond & Midgley 2000, Hoffmann *et al.* 2003, Hoffmann & Solbrig 2003, Prior *et al.* 2004, Prior *et al.* 2006). However, most studies on the role of reserves during resprouting have been in Mediterranean-type shrublands (Bowen & Pate 1993, Bell & Pate 1996, Canadell & Lopez-Soria

1998) with few in savannas (Miyanishi & Kellman 1986, Hoffmann *et al.* 2000). Resprouting of woody species is generally associated with declining root starch concentration. One year after a single fire, the root starch concentration of resprouting *Miconia albicans* and *Cildemia sericea,* two neotropical savanna shrubs, were 30 percent depleted compared to unburnt plants (Miyanishi & Kellman 1986). Root starch concentrations of *Stirlingia latifolia,* a Mediterranean-type shrub growing in South West Australia, decreased by 50 to 75 percent during the first 2 to 5 months after topkill (Bowen & Pate 1993). If plants are topkilled when root starch reserves are already low, such as can happen after repeated disturbance or soon after leaf flush, plants resprout poorly (Jones & Laude 1960; Landhäusser & Lieffers 2002) or not at all (Bowen & Pate 1993; Miyanishi & Kellman 1986). Neither the neotropical savanna shrubs (Miyanishi & Kellman 1986) nor the Mediterranean shrub (Bowen & Pate 1993) resprouted if they were topkilled when their root reserves were less than 10 percent of pre-disturbance levels.

Whether root reserves of carbohydrates are important for growth and survival of resprouting plants will depend on their contribution of carbon to these processes relative to the contribution of concurrent photosynthesis (Danckwerts 1993; Richards & Caldwell 1985). Although root reserves decrease after topkill, this decrease might contribute a small proportion of the energy and substrate requirements of the plant during recovery if leaf area can be recovered quickly at relatively little cost. For example, carbon contributed to shoot growth and nocturnal respiration by concurrent photosynthesis exceeded that contributed by carbon reserves only 3 days after clipping in severely defoliated *Agropyron* grasses (Richards & Caldwell 1985). Root starch contributed less than 10 percent to new shoot biomass of resprouting *Eucalyptus kochii,* a western Australian shrub, during the first year after topkill (Wildy & Pate 2002). However, a small overall contribution of reserves to new biomass might belie the importance of reserves in supplying the carbon required for rapid foliage recovery, in the initial stages of resprouting (Danckwerts 1993; Heilmeier *et al.* 1986).

This study aimed (1) to compare concurrent photosynthesis and root storage as sources of carbon during resprouting of *Acacia karroo* Hayne, (2) to establish whether the carbon incorporated into new aboveground biomass of topkilled plants could be accounted for by concurrent photosynthesis, and (3) to compare the photosynthetic capacity of burnt resprouting plants and unburnt plants. To quantify concurrent photosynthesis as a source of carbon, a model was developed that estimated the net daily photosynthetic rate (gC day^{-1} m^{-2}) of plants for each day for a year after topkill. In the model, photosynthetic rates were estimated based on light saturated photosynthetic rates, light response curves, and light levels, which were all measured empirically. The daily carbon gain was calculated as the product of net daily photosynthetic rate and whole plant leaf area.

Methods

Study site and species

The fieldwork for this study was conducted in a 1 ha fenced enclosure (S 28.20161°, E 32.04218°, elevation 140m) in the Gunjaneni area on the eastern border of the Hluhluwe-iMfolozi Park (HiP). Hluhluwe–iMfolozi Park is a 900 km^2 fenced nature reserve in KwaZulu–Natal, South Africa. The vegetation of the park is predominantly fine-leaved Acacia savanna (Whateley & Porter 1983). The park has a mean annual rainfall ranging from 990mm in the north western hills to less than 635 mm in the low lying southern regions (Balfour & Howison 2001). The majority of the rain falls during summer thunderstorms (Balfour & Howison 2001). Between 1956 and 1996, the mean fire return interval for the park was 4 years, whereas the median fire interval was 1.3 years (Balfour & Howison 2001). Approximately 90% of the fires recorded over this period burnt between June and September (i.e. in the dry season) (Balfour & Howison 2001).

The mean (±SE) rainfall between June 2005 and July 2006, recorded at three weather stations all within 5 km of Gunjaneni, was 507 ± 70 mm (Zululand Tree Project, University of Cape Town). At these three sites, 78 ± 2 % of the rain fell between October 2005 and March 2006. Between 1956 and 2005, the mean fire interval at Gunjaneni was 3.8 years. Within the enclosure the soils were dark brown, slightly acidic (pH 5.5), fine textured, and derived from dolerite parent material (Oakleaf & Glenrosa) (HiP Research Centre, Soils Map). The tree component in the enclosure was dominated by *Acacia karroo* adults and juveniles, and *Acacia nilotica* adults.

The shrub layer was dominated by *Acacia caffra, Dichrostachys cinerea* and *Maytenus heterophylla*. The grass sward was dominated by *Cymbopogon excavatus, Eragrostis curvula, Themeda trianda,* and *Panicum deustum*. For this study, half of the plot was burnt on the 30th August, 2005, so as to allow for the comparison of unburnt and burnt resprouting plants. Before the 2005 fire, the plot had last burnt in 2001.

A. karroo is generally noted as one of the key species responsible for bush thickening in southern African savannas (Hoffman & O'Connor 1999; Hoffman *et al.* 1999; O'Connor 1995; Skowno *et al.* 1999). The Hluhluwe form of *A. karroo* is particularly abundant in tall fire-prone grasslands (Bond *et al.* 2001). *A. karroo* typifies the life history strategy described by Bond and van Wilgen (1996) and Gignoux *et al.* (1997), whereby juvenile savanna trees survive frequent topkill events, by repeatedly sprouting from their fire-protected rootstock.

Study design

The photosynthetic rates and leaf area of unburnt and burnt resprouting plants were recorded once every 3 months for one year after the August 2005 fire. These spot measurements were then used to interpolate total carbon assimilation per plant for the entire season. The carbon investment in shoot biomass for each of the resprouting plants was also modelled for 1 year after the fire. The roots of the burnt plants were harvested 1 year after the fire and analysed for starch content.

Gas exchange measurements

Daily gas exchange patterns

Light saturated photosynthetic rates (A_{max}), transpiration rate (E), stomatal conductance (G_s), and the ratio of intercellular CO_2 concentration and ambient CO_2 concentration (C_i:C_a) of leaves of unburnt and resprouting burnt plants were measured four times between October 2005 and August 2006. The water use efficiency (WUE) of the leaves was calculated by dividing the photosynthetic rate of the leaves by the transpiration rate of the leaves. On each of the sampling days, readings were taken at *ca.* 2 h intervals, starting at 09:00 and ending at 17:00, using an Infrared Gas Analyser

(IRGA) (LI-6400 portable photosynthesis system, LI-COR, Lincoln, Nebraska, USA). Measurements were taken on sunlit leaves that were fully expanded and located near the apex of the shoot or branch. The same leaf on each plant was used throughout the day. The area of leaf that was inside the cuvette was calculated for each leaf by scanning the leaves and analysing the images in Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, California, USA). All the plants sampled had a known pre-fire height of between 1 to 2 m Dark respiration rates were recorded, at least 3 h into the dark period, on each of the sampling dates. For the measurements taken over the length of a day, the concentration of CO_2 in the reference chamber was set to 400 ppm, the flow rate was set to 500 μmol $^{s-1}$. The relative humidity in the cuvette and the leaf temperature of the measured leaf were allowed to vary with the ambient conditions. The light level inside the cuvette of the IRGA was set to 1250, 1500, 2000, and 1500 μmol m^{-2} s^{-1} in October 2005, January 2006, May 2006, and August 2006, respectively. These values were the light saturation points of the leaves, in the different months, as recorded by plotting light response curves.

Light response measurements

In each of the sampling months, light response curves were plotted by taking photosynthetic rate measurements on a single leaf at various light levels, ranging from 0 to 2500 μmol m^{-2} s^{-1}, while keeping other variables, such as the temperature and the CO_2 concentration inside the leaf chamber, constant. The temperature inside the cuvette was kept at the ambient temperature recorded at the start of the readings, which was generally between 26 and 28° C. At each light level, the leaf was allowed to acclimate for a minimum of 2 minutes and a maximum of 3 minutes. These measurements were taken between 9:00 and 12:00 in the morning, on no less than three plants.

Stem biomass, leaf biomass, and leaf area measurements

Changes in the leaf area of burnt and unburnt plants were tracked for *ca.* one year after the August 2005 fire. The leaf area of the plants was calculated as a function of the total leaf mass of the plants (Resprouting plants: leaf area (cm^2) = 68.7×leaf mass (g, R^2=0.91, n=22, p<0.001; Unburnt plants: leaf area (cm^2) = 48.9×leaf mass (g), R^2=0.92, n=25, p<0.001).

The stem and leaf mass of the burnt resprouting plants were determined allometrically based on stem volume. Stem volume was calculated as a product of stem basal area and stem length divided by two, which is the formula for the volume of a paraboloid. The stem diameter and length of the each of the coppicing shoots of the trees were recorded in October 2005, January 2006, April 2006, and August 2006. To determine the allometric relationship between stem volume and stem and leaf mass, additional shoots were measured, harvested, separated into stem and leaf material, dried at 70°C for 72 h, and weighed in January, April, and August 2006 (Fig. 5.1, Table 5.1). The October 2005 stem and leaf mass estimates were made using the January 2006 regression equations. The pre-fire size of the resprouting plants was determined by measuring the height and the diameter of the dead stem in September 2005.

The total leaf mass of unburnt saplings was determined by analysing digital photographs of the plants using Adobe Photoshop 7.0 (Adobe Systems Inc., USA). The photographs were taken at the same intervals as coppicing plants were measured. The number of pixels in each photograph that represented leaves was calculated in Photoshop 7.0 and this value was converted to leaf mass using a regression equation. The regression equation was based on photographs of trees, between 0.5m tall to 2m tall, whose leaves had been harvested directly after taking the photographs, dried at 70°C for 72 h, and weighed (R^2=0.93, n=19, p<0.001).

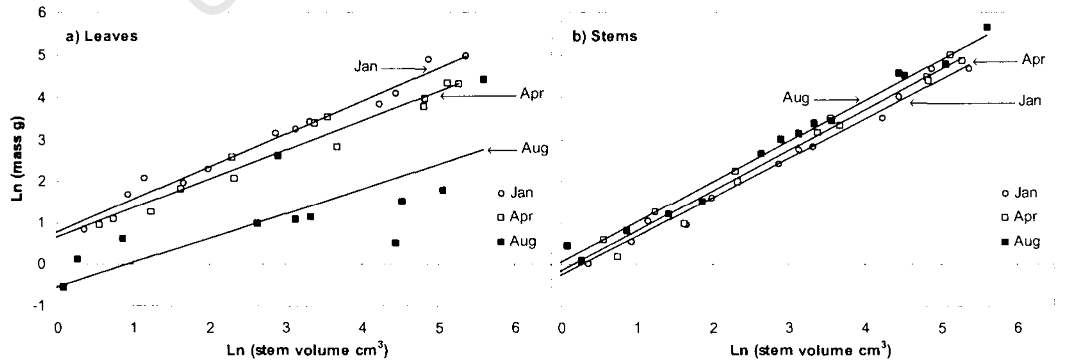

Fig. 5.1. The relationship between stem volume and (a) leaf mass and (b) stem mass in January, April and August 2006 of the burnt plants. Stem volume was calculated as a product of stem basal area and stem length divided by two, which is the formula for the volume of a parabola. The regression equations and the correlation coefficients are shown in Table 5.1.

Table 5.1. The regression equations determined to calculate stem and leaf mass (g) of the burnt plants using the volume (cm^3) of the stem at different times of the year.

Month	Organ	Regression	R^2	F value	P value
Jan-06	leaves	$y = 2.21x^{0.78}$	0.98	$F_{1,11} = 552$	p = 0.0000
	stem	$y = 0.78x^{0.93}$	0.99	$F_{1,11} = 787$	p = 0.0000
Apr-06	leaves	$y = 1.95x^{0.69}$	0.97	$F_{1,13} = 429$	p = 0.0000
	stem	$y = 0.84x^{0.97}$	0.99	$F_{1,13} = 1103$	p = 0.0000
Aug-06	leaves	$y = 0.21x$	0.67	$F_{1,12} = 30.7$	p = 0.0002
	stem	$y = 1.11x^{0.96}$	0.99	$F_{1,11} = 1015$	p = 0.0000

Carbon assimilation and allocation to shoot biomass

Photosynthesis model

The leaf area and A_{max} data, along with data on photosynthetically active radiation (PAR) levels, to estimate the amount of carbon assimilated, for approximately 1 year after the August 2005 fire, by the burnt resprouting plants and the unburnt plants. The leaf area of each plant was estimated for each day, from the start of September 2005 to end of August 2006, by linearly interpolating between the leaf area measurements made on the sampling dates. Assimilation rates were estimated based on the A_{max} readings, a light response model, and solar radiation readings taken at half-hourly intervals. The data collected for the light response curves were used to parameterise the light response model, which was based on the simple rectangular hyperbola model given by Charles-Edwards *et al.* (1986) and by Thornley (1976)

$$A = \alpha I\, A_{max}/(\alpha I + A_{max}) - R_D \qquad \text{Eqn 1}$$

where A is the net rate of leaf photosynthesis, I is the irradiance or PAR incident on the leaf, α is the leaf quantum yield, A_{max} is the rate of light saturated leaf photosynthesis, and R_D is the dark respiration rate of the leaf. The parameters were determined using a least squares minimisation method provided by CurveExpert 1.3 (Hyams Development, Tennessee, USA). To standardise for the difference in A_{max} across the seasons, the values used to parameterise the model were expressed as a proportion of A_{max} for the particular season. The model returns A as a proportion of A_{max}, referred to as $A_{\%max}$ hereafter, i.e.

$$A_{\%max} = \alpha I\, A_{max1}/(\alpha I + A_{max1}) - R_D \qquad \text{Eqn 2}$$

Where A_{max1} is the maximum value that the model can predict for $A_{\%max}$, which should be close to one. A_{max}, $A_{\%max}$ and A were calculated for each half hour interval for each day from the 1st September, 2005, to the 28th August, 2006. A_{max} was calculated using the light saturated photosynthetic rates recorded in the field. A_{max} was interpolated linearly between readings on a particular day and between readings taken in different seasons. $A_{\%max}$ was calculated using Eqn 1 and A was calculated as the product of $A_{\%max}$ and A_{max}. Dark respiration at night was approximated to the actual dark respiration values recorded in the field. The total amount of CO_2 m^{-2} fixed was calculated for each half hour (gCO_2 m^{-2} s^{-1} ×30 min × 60 sec) and then summed up for each day to calculate the net daily photosynthetic rate (gCO_2 m^{-2} day^{-1}). The daily amount of CO_2 fixed by each plant (gCO_2 day^{-1}) was estimated by multiplying the net daily photosynthetic rate by the estimated leaf area for the specific day (Danckwerts 1993). The daily amount of CO_2 assimilated by each plant was summed to give an estimate of the total amount of CO_2 assimilated by each plant between the 1st September, 2005, and the 28th August, 2006.

The PAR levels used in the model were calculated from solar radiation (watts m^{-1}) data recorded at half-hour intervals from September 2003 to September 2004 with a solar radiation sensor (Davis Weather Pro, Davis Instruments, Hayward, California, USA) *ca.* 5 km from the study site. Solar radiation was converted to Photosynthetically Active Radiation (PAR) by multiplying solar radiation by the percent of radiation that is in the PAR spectrum (45%) and the conversion factor between watts m^{-2} and μmol m^{-2} s^{-1} (4.6) (Kirk Baker, Lake Michigan Air Directors Consortium).

The model used in this study had a number of implicit assumptions. For example, self shading was not accounted for, however, the coppicing shoots of resprouting *A. karroo* radiate out from the root crown and rarely shade each other. Leaf age was not explicitly accounted for, however, leaf age is fairly constant across the canopy as, in the absence of browsing or fire, *A. karroo* typically produces leaves in a single flush at the start of the wet season. Perhaps the largest caveat of the model was that soil moisture effects were not accounted for explicitly. However, by taking empirical measurements in the dry season and in the wet season at least some of these effects would have been implicit within the model.

Leaf and stem carbon concentrations and pools

Carbon investment in shoot biomass was calculated as the sum of the carbon invested in stem and leaf biomass. The carbon invested in stem and leaf biomass was calculated as the product of the stem or leaf biomass and the mean concentration of carbon in the stem or leaf tissue, respectively. The carbon concentration in leaf and stem tissues of coppicing *A. karroo* were determined using a Thermo Flash EA 1112 series elemental analyser (Thermo Electron Corporation, Milan, Italy). The dried leaf and stem samples were milled in a Wiley mill (Arthur H. Thomas Co., Philadelphia, USA) with a 1 mm sieve-plate. Sub-samples of between 2.1 and 2.2 mg were weighed into 8 × 5 mm tin capsules (Elemental Microanalysis, Devon, UK) and combusted in the elemental analyser. The stem and leaf mass of the each of the resprouting plants was estimated for each day for one year after the August 2005 fire by linearly interpolating between the four measurements taken between October 2005 and August 2006. The amount of new carbon invested in shoot biomass was calculated for each day by multiplying the growth in stem or leaf biomass for the day by the concentration of carbon in the stem or leaf tissue.

Model sensitivity

To determine the sensitivity of the photosynthesis model, A_{max} and leaf area were varied from 12.5% to 200% of the recorded values. To determine whether the output of the model was more sensitive to A_{max} or leaf area, A_{max} was changed while leaf area was kept at the original values and visa versa. To determine whether the model output was sensitive to changes in particular months, A_{max} and leaf area were varied for a particular period but kept at their original values for the other months.

Root starch analysis

The roots of the 30 coppicing plants whose canopy areas were monitored were harvested in the last week of August 2006. Thirty centimetres of the root, measured from the lowest stem scar, was excavated. Immediately after returning from the field the roots were dried at 70°C for 5 days and then weighed. The roots were milled using a Wiley mill (Arthur H. Thomas Co.) with a 1 mm sieve-plate. Starch in sub-samples (50 mg) of dried, ground root material was hydrolysed to glucose using the amyloglucosidase–alpha-amylase method described by McCleary *et al.* (1997). The glucose concentration of the resulting solution was determined colorimetrically using the phenol–sulphuric method described by Dubois *et al.* (1956). Prior to the starch determination, soluble sugars were removed from the sub-sample by washing the sub-sample in hot (80°C) 80% ethanol (v/v) for 5 minutes, centrifuging the solution at 760 × *g* for 10 minutes, and discarding the supernatant. This process was repeated three times. Starch was hydrolyzed to glucose by incubating the residue remaining after the sugar extraction for 5 minutes at 100°C with 300 U thermo-stable alpha-amylase (from *Bacillus lichen*, Sigma-Aldrich (Pty) Ltd, Castle Hill, New South Wales, Australia); and then incubating the solution for 1 h at 55°C after adding 20 U purified amyloglucosidase (from *Aspergillus niger*, Sigma-Aldrich). The hydrolysate was centrifuged at 760 × *g* for 10 minutes, and the supernatant was used in the colorimetric procedure. For the colorimetric procedure, 200 µl of 5% phenol was added to 200 µl of sample, and 1 ml of concentrated sulphuric acid was added to the mixture. The colour was allowed to develop for at least 15 minutes before the absorbance was read at 490 nm with a microplate spectrophotometer (PowerWave XS, BioTek, Winooski, Vermont, USA).

Results

Gas exchange measurements

Gas exchange and light response curves

Pairs of gas exchange measurements taken for the burnt and the unburnt plants in the same month were generally different in October 2005, but thereafter the gas exchange properties of the burnt and unburnt plants followed similar trends (Fig. 5.2). The light saturated photosynthetic rate (A_{max}) ($F_{1,256}=7.4$, $p=0.007$), the transpiration rate ($F_{1,256}=33.5$, $p<0.001$), the stomatal conductance ($F_{1,256}=4.0$, $p=0.047$), and the C_i:C_a ratio ($F_{1,256}=15.9$, $p<0.001$) of the burnt plants were significantly larger than that of the unburnt plants in October 2005, but not significantly different from that of the unburnt plants after October 2005. The WUE of the burnt plants was significantly less than that of the unburnt plants in October 2005 ($F_{1,256}=9.7$, $p = 0.002$), but not significantly different from the WUE of the unburnt plants after October 2005. A_{max}

increased between October and January and declined between January and August, whereas the transpiration rate of the plants stayed fairly constant. The stomatal conductance of the leaves also declined between January and August, while the C_i:C_a ratio increased slightly. The WUE efficiency of the plants increased as the wet season progressed, i.e. between October and May, but decreased again in the dry season proper. The dark respiration rates of the leaves on the resprouting plants were significantly higher than that of the leaves on the unburnt plants in October 2005 ($F_{1,44}$=20.5, $p<0.001$), but similar to that of the leaves on the unburnt plants after October 2005 (Table 5.2). The dark respiration rates of the leaves of the burnt and unburnt plants decreased between October 2005 and August 2006.

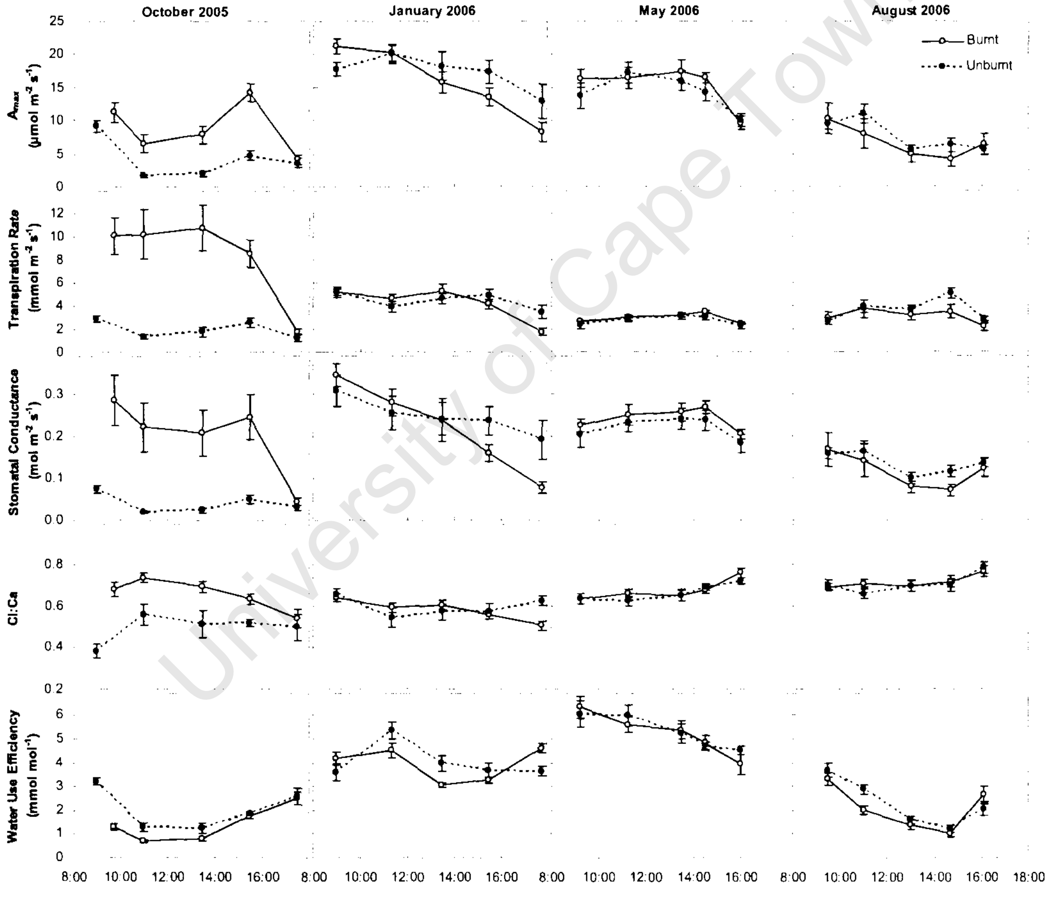

Fig. 5.2. Mean (±SE) gas exchange measurements for burnt resprouting and unburnt plants between October 2005 and August 2006, after the August 2005 fire.

Table 5.2. The mean (±SE) dark respiration rates (μmol m^{-2} s^{-1}) of burnt and unburnt plants between October 2005 and August 2006.

Month	Burnt	Unburnt
Oct-05	3.9 ± 0.60	1.2 ± 0.28
Jan-06	1.0 ± 0.09	0.7 ± 0.20
May-06	0.3 ± 0.03	0.3 ± 0.11
Aug-06	0.5 ± 0.07	0.4 ± 0.05

Leaf and stem biomass of the resprouting plants

The leaf and stem biomass of the resprouting plants were significantly correlated to the pre-fire height of the plants on all four of the sampling dates ($p < 0.001$) (Fig. 5.3). The slopes of the regression equations that predicted leaf biomass as a function of pre-fire height were significantly steeper than the slopes predicted by the regression equations for stem biomass in October 2005 ($F_{1,40}$=7.3, p = 0.01) and in January 2006 ($F_{1,40}$=7.9, p = 0.008), but not in April 2006 (Fig. 5.3). By August 2006, the plants had dropped most of their leaves and the slope of the regression equation that predicted stem biomass as a function of pre-fire height was significantly steeper than the slope predicted for leaf biomass ($F_{1,40}$=51, $p < 0.001$). During the first growing season after topkill, the mean ratio of leaf to stem biomass of the resprouting plants decreased steadily from 3.2 ± 0.1, in October 2005, to 1.3 ± 0.1, in April 2006. By August 2006, the ratio of leaf to stem biomass of the resprouting plants had fallen to 0.2 ± 0.002.

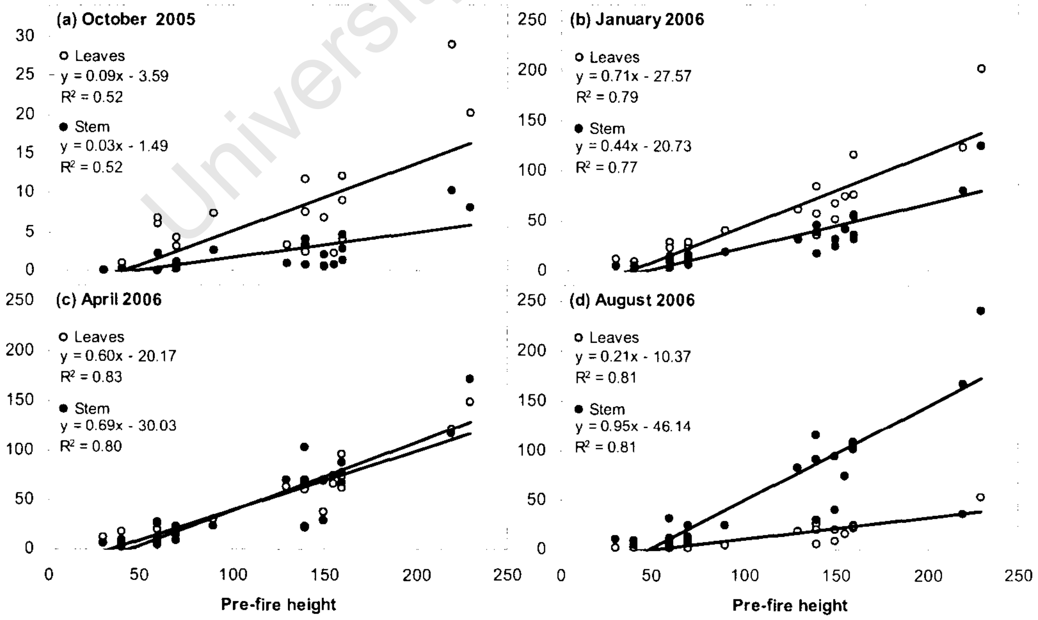

Fig. 5.3. The leaf and stem biomass of burnt resprouting plants in (a) October 2005, (b) January 2006, (c) April 2006, and (d) August 2006. The leaf and stem biomass of the plants were significantly correlated to pre-fire height of the plants on all four of the sampling dates ($p < 0.001$).

Leaf Area

The leaf area recovery rate of the resprouting trees was strongly and positively correlated to the pre-fire height of the trees (Fig. 5.4). By January 2006, there was no significant difference in the total leaf area of burnt and unburnt plants that were a similar size before the August 2005 fire (ANCOVA, $F_{1,42}$=0.8, p = 0.4). There was also no significant difference in the leaf area of burnt and unburnt trees, after controlling for pre-fire size, in April 2006 (ANCOVA, $F_{1,42}$= 0.3, p = 0.6) or in August 2006 (ANCOVA, $F_{1,32}$= 0.7, p = 0.4). The total leaf area of the plants followed a seasonal pattern with maximal values recorded between January and April, i.e. from the middle of summer into the start of the dry season, and minimal values recorded in August, i.e. the middle of the dry season.

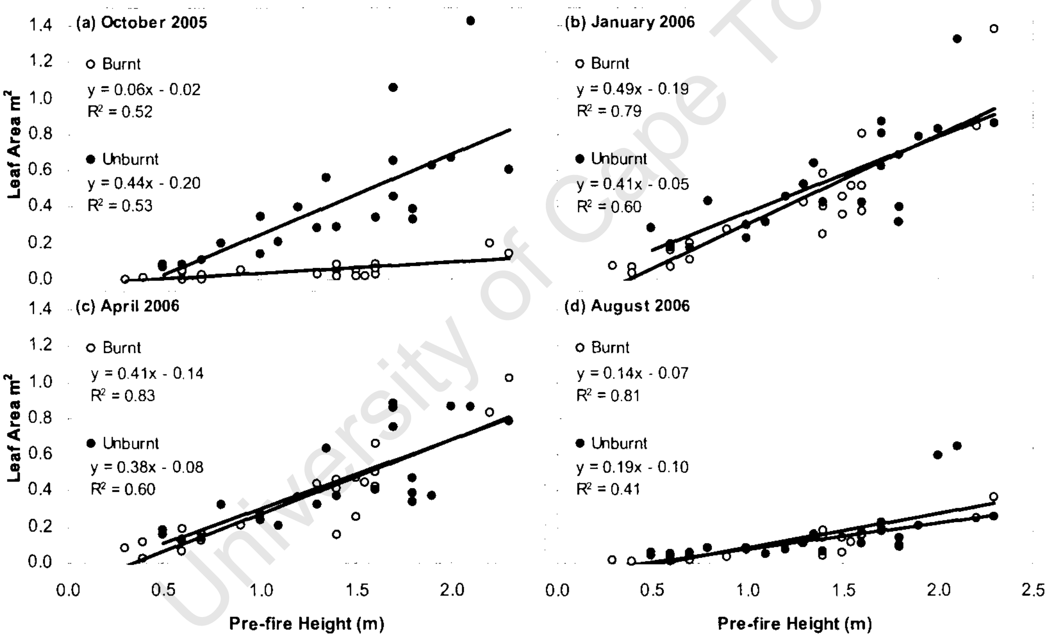

Fig. 5.4. The leaf area of the burnt and unburnt plants in (a) October 2005, (b) January 2006, (c) April 2006, and (d) August 2006. Variation in leaf area was significantly correlated ($p < 0.001$) to the pre-fire height of the plants on all four sampling dates.

Carbon assimilation and allocation to shoot biomass

Photosynthesis model

The maximum net daily assimilation rates (gCO_2 m^{-2} day^{-1}) of the unburnt and burnt plants were similar on all sampling dates ($F_{1,57}$=0.02, p=0.9) (Fig. 5.5a), even in October 2005. Although the diurnal instantaneous A_{max} readings (μmol m^{-2} s^{-1}) of the burnt plants were generally higher than that of the unburnt plants in October 2005 (Fig. 5.2), the respiration rates of the burnt plants at this time were higher than that of the unburnt plants (Table 5.2), which explains their similar net daily assimilation rates. A_{max} peaked in late January, similar to total leaf area, however, unlike leaf area (Fig. 5.4), A_{max} declined between January and March concurrently with Photosynthetically Active Radiation (PAR) (Fig. 5.5b). PAR levels followed a typical sinusoidal seasonal pattern with maximum levels over the summer and minimum levels over the winter.

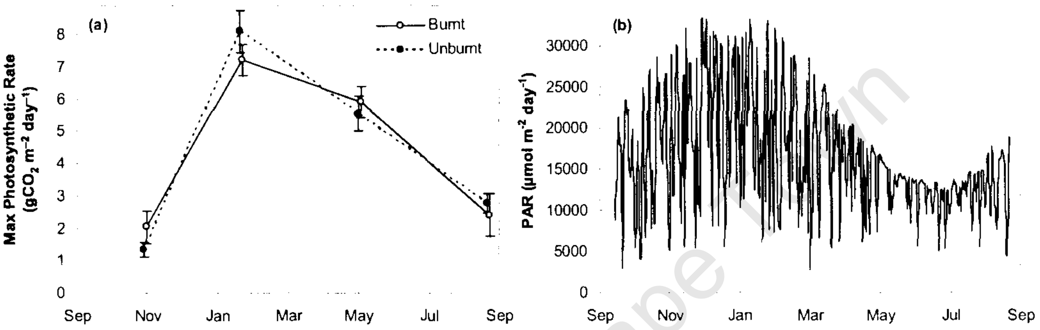

Fig. 5.5. (a) The mean (±SE) maximum net daily photosynthetic rate, including leaf respiration, of the burnt and unburnt plants between October 2005 and August 2006. (b) The 2003–2004 Photosynthetically Active Radiation (PAR) data. (Note that the maximum photosynthetic rate and PAR are given in the figures on a per day basis for illustrative purposes only; in the photosynthesis model, the values were calculated for each half hour interval of the day.)

The rectangular hyperbola equation modelled the light response data very effectively ($R^2 = 0.99$, $n = 127$, $p < 0.001$) (Fig. 5.6). The least squares minimisation method determined the leaf quantum yield (α) to be 0.0029, $A_{max l}$ to be 1.4, and R_D to be 0.16 (see Eqn 2). One would expect $A_{max l}$ to be closer to 1, and the relatively high value determined for $A_{max l}$ is an artefact of the relatively simple model used (Eqn 2). Nevertheless, using these values $A_{\%max}$ is still only equal to 1.01 when PAR is 2500 μmol m^{-2} s^{-1}, which is unlikely to be exceeded in the field.

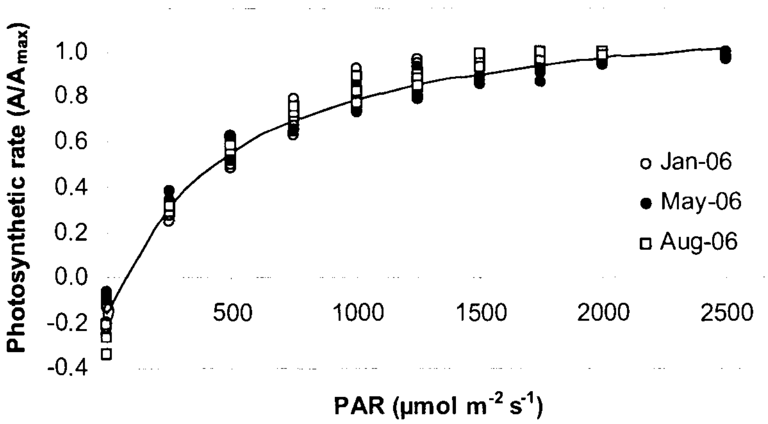

Fig. 5.6. The light response curve of fully sunlit leaves on coppicing *A. karroo* individuals. To account for seasonal differences, the data from the different seasons was standardized as a proportion of the A_{max} recorded for the particular season The points were fit to a rectangular hyperbola model $A_{\%max} = \alpha I\ A_{max1}/(\alpha I + A_{max1}) - R_D$ (see text for explanation), where $\alpha = 0.0029$; $A_{max1} = 1.4$; $R_D = 0.16$.

The mean net daily photosynthetic rates ($gCO_2\ m^{-2}\ day^{-1}$) of the burnt and unburnt plants, as calculated by the photosynthesis model, were similar throughout the year (Fig. 5.7a & b), both peaked in late January when leaf area, A_{max}, and PAR were maximal and declined as the dry season progressed. The mean amount of carbon assimilated per day by the burnt plants was lower than that of the unburnt plants (Fig. 5.7c & d), particularly in the first 3 months after topkill when the leaf area of the burnt plants was still very low. In the middle of the growing season, the peak assimilation rate per day of burnt plants was approximately two-thirds that of the unburnt plants.

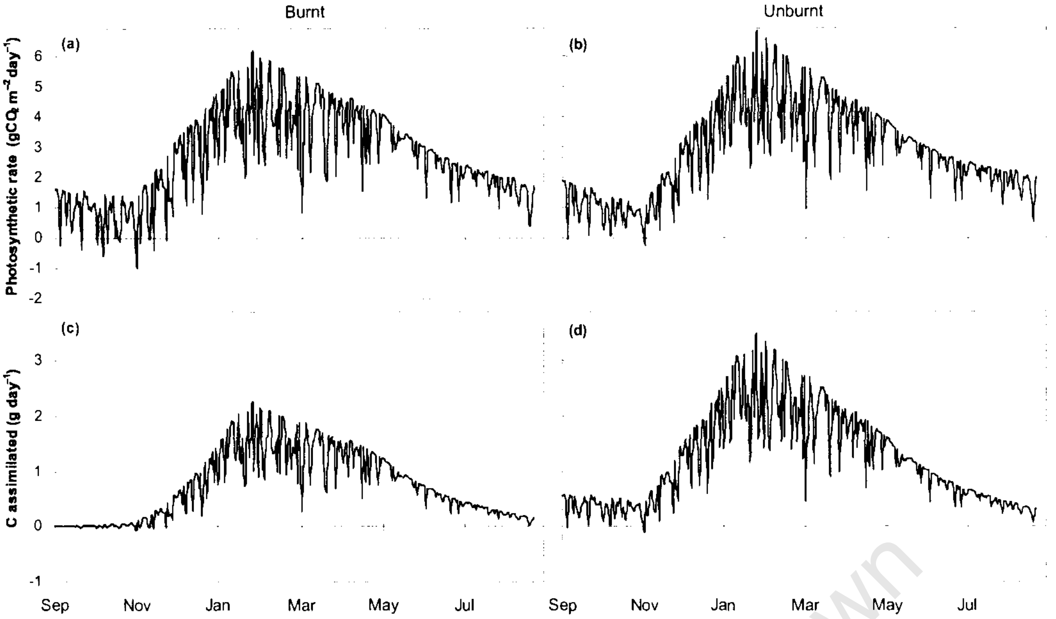

Fig. 5.7. The mean net daily photosynthetic rate of (a) the burnt and (b) the unburnt plants as calculated from the A_{max} data, the PAR data, and the light response model. The mean amount of carbon assimilated per day by (c) the burnt and (d) the unburnt plants as calculated from the net daily photosynthetic rate and leaf area of the plants.

One year after topkill, the total amount of carbon assimilated by both the burnt ($R^2 = 0.82$, $n = 22$, $p < 0.001$) and the unburnt ($R^2 = 0.63$, $n = 23$, $p < 0.001$) plants was significantly correlated to the pre-fire size of the plants (Fig. 5.8). After controlling for variation caused by pre-fire height, the mean amount of carbon assimilated by the burnt plants was significantly lower than that of the unburnt plants (ANCOVA, $F_{1,42} = 4.5$, $p = 0.04$). According to the regression equations, a tree 1.25 m tall before the fire would have assimilated *ca.* 380 g C during the year after the fire if it had not been burnt and *ca.* 300 g C if it had been burnt.

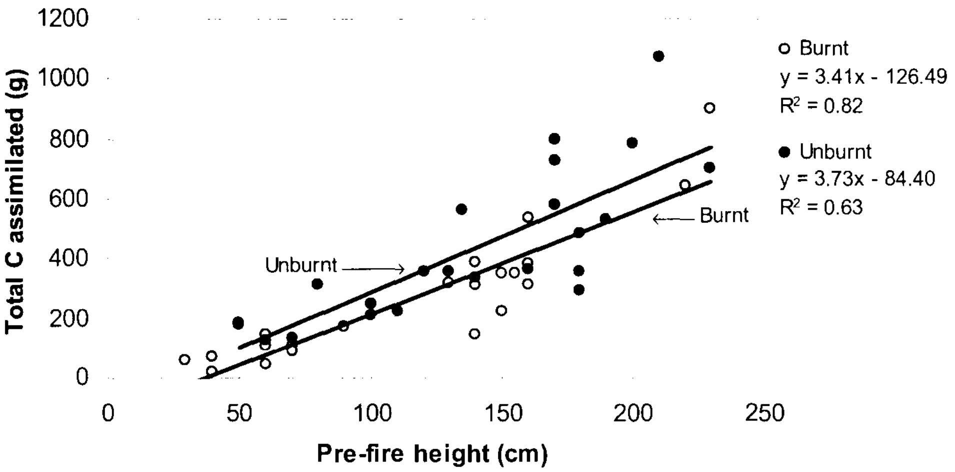

Fig. 5.8. The total carbon assimilated by the burnt and unburnt plants over one year after the August 2005 fire. The carbon assimilated was significantly correlated ($p < 0.001$) to pre-fire height for both the burnt and the unburnt plants.

Assimilated Carbon, Shoot Carbon and Root Starch

The measured carbon concentration in the stem and leaves of resprouting *A. karroo* saplings was 43.0% ± 0.4% and 50.0% ± 0.3%, respectively. According to the photosynthesis model, during the first 3 months (or 92 ± 1 days) after topkill, shoot growth in g C per day was greater than the amount of carbon assimilated in the day (Fig. 5.9a). In August 2006, the mean measured starch concentration in the roots of the burnt plants was 36% ± 1% of dry weight. This concentration and the mass of the excavated roots were used to calculate a proxy of pre-fire starch pools of the burnt plants. The carbon growth of the shoots between September 2005 and December 2006 (Fig. 5.9a) that was unaccounted for by photosynthesis, represented 65% ± 8% of the root carbohydrates reserves and 26% ± 1% of the total carbon incorporated into shoot biomass. From January onwards, the daily amount of carbon assimilated exceeded the daily investment of carbon into shoot biomass. By the end of August 2006, i.e. 1 year after fire, the carbon incorporated into shoot biomass accounted for only 19% ± 1% of the total assimilated carbon (Fig. 5.9b).

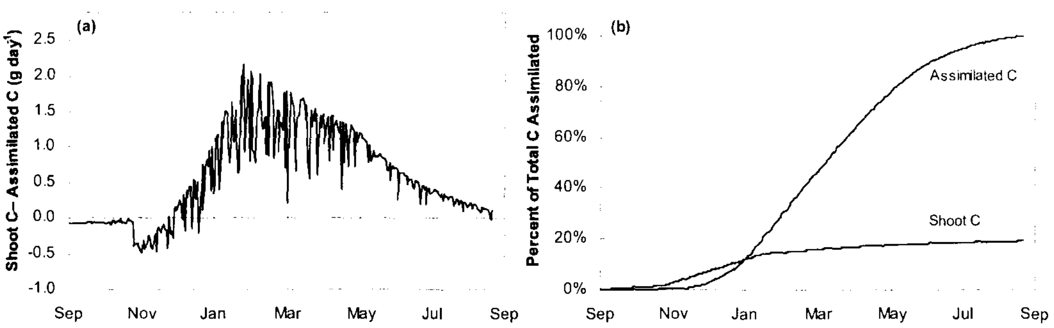

Fig. 5.9. (a) The mean (±SE) difference in shoot growth per day, expressed as g C day^{-1}, and carbon assimilated per day by the burnt resprouting plants for one year after the August fire. (b) The total amount of carbon invested in shoot biomass of the resprouting plants shown as a percentage of the total carbon assimilated over the year.

One year after the fire, the amount of carbon assimilated by the burnt plants, incorporated into shoot biomass, and stored as starch in the taproots of the resprouting plants were all positively correlated to the pre-fire height of the plants (Fig. 5.10). However, the slope of the shoot carbon curve was significantly less than that of assimilated carbon ($F_{1,40} = 53$, $p < 0.001$); and the slope of the root starch carbon curve was significantly less than that of the shoot carbon curve ($F_{1,40} = 57$, $p < 0.001$). According to the regression equations, a plant that was 1.25 m tall before the fire, and was burnt in the fire, would have assimilated *ca.* 300 g C, incorporate *ca.* 60 g C into shoot biomass, and would have been able to store *ca.* 15 g C in its taproot during the year after the fire.

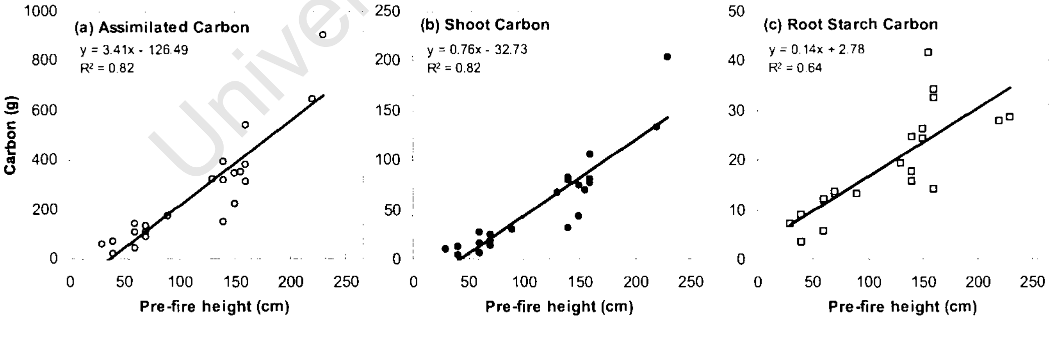

Fig. 5.10. (a) The total amount of carbon assimilated by the resprouting plants one year after the August 2005 fire, (b) the carbon invested in new shoot biomass by the resprouting plants one year after the fire, and (c) the amount of carbon stored as starch in the taproots of the resprouting plants. Assimilated carbon, shoot carbon, root starch carbon were all significantly correlated (p < 0.001) to pre-fire height

Model sensitivity

In terms of the ratio of assimilated carbon to shoot carbon and the percent of the year when more carbon had been assimilated than was invested in shoot carbon, the model output was sensitive to changes to the January and May 2006 A_{max} values and the January and April 2006 leaf area values (Fig. 5.11). With the original values of A_{max} and leaf area (i.e. at 100%), assimilated carbon was 5-fold the amount of shoot carbon 1 year after the fire (Fig. 5.11a). If the January or May 2006 A_{max} values were doubled, assimilated carbon was more than 7-fold the amount of shoot carbon. If the January or May 2006 A_{max} values were halved (i.e. 50%), assimilated carbon was still at least 3-fold the amount of shoot carbon. The assimilated carbon to shoot carbon ratio was not sensitive to increases in leaf area above what was originally used in the model, but it was sensitive to decreases in leaf area (Fig. 5.11b). However, even if the January or April 2006 leaf area values were halved, assimilated carbon was still more than 3-fold the shoot carbon. With the original values of A_{max} and leaf area, the accumulated amount of assimilated carbon was greater than the amount of shoot carbon for *ca.* 65% of the year after the fire. If the October 2005 or January 2006 A_{max} values were doubled, assimilated carbon was greater than shoot carbon for *ca.* 70% of the year (Fig. 5.11c). If October 2005 or January 2006 A_{max} values were halved, assimilated carbon was still greater than shoot carbon for *ca.* 60% or *ca.* 55% of the year, respectively. The percent of the year that accumulated assimilated carbon was greater than shoot carbon was sensitive to changes in the January 2006 leaf area (Fig. 5.11d). However, even if the January 2006 leaf area was doubled or halved, assimilated carbon was still greater than shoot carbon for *ca.* 65% of the year.

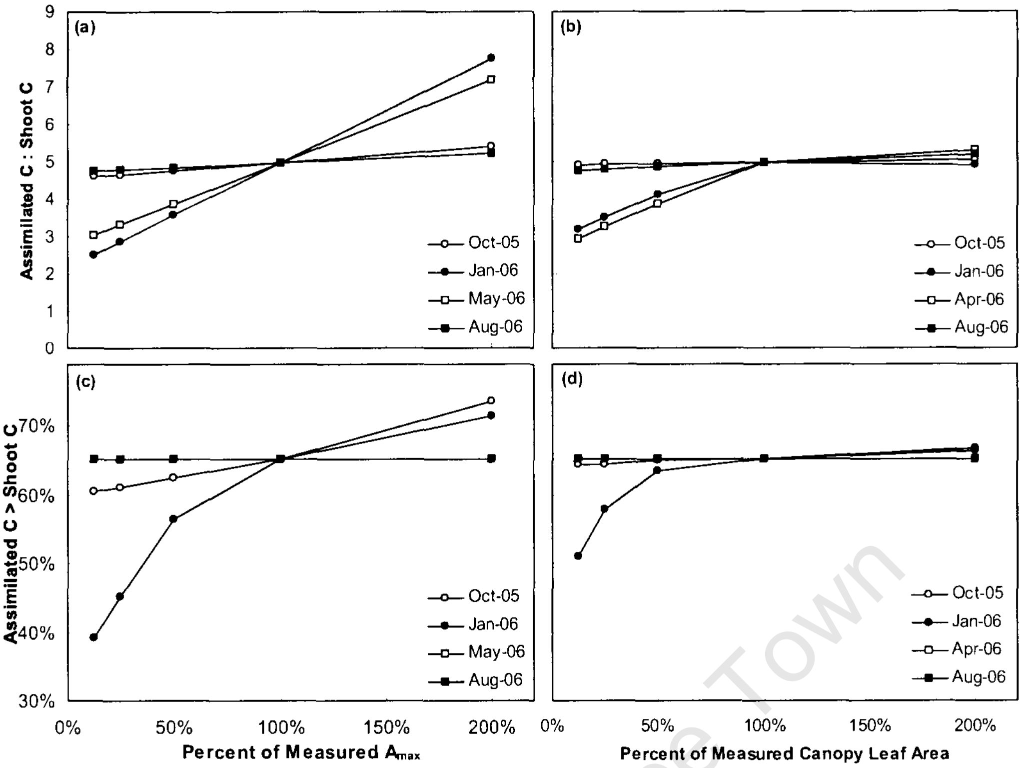

Fig. 5.11. The sensitivity of the photosynthesis model to changes in A_{max} (a and c) and leaf area (b and d) at different times of the year. The sensitivity of the model was investigated in terms of the ratio of assimilated carbon to shoot carbon (a and b) and the percent of the year when accumulated assimilated carbon was greater than that invested in shoot carbon (c and d).

Discussion

The light saturated photosynthetic rate (A_{max}) and the total leaf area of both the burnt and the unburnt plants peaked in January, stayed fairly high into the early dry season, i.e. May, but then declined to minimum levels as the dry season progressed (Fig. 5.2 & Fig. 5.5a). In October 2005, just 1.5 months after the fire, A_{max} of the burnt plants was relatively high compared to the unburnt plants, which is similar to the pattern reported for burnt and unburnt trees in Australian savannas (Cernusak *et al.* 2006) and north American prairies (McCarron & Knapp 2003). The difference in the A_{max} of burnt and unburnt plants could be the result of improved resource availability as a result of the decreased grass sward and the input of N and P in the form of ash (Cernusak *et al.* 2006, McCarron & Knapp 2003). Or it could be a consequence of the increased root:shoot ratio of the burnt plants, and thus increased resource availability per unit leaf (McCarron & Knapp 2003; Tschaplinski & Blake 1989). Or it could be a consequence of different leaf age as photosynthetic potential can decrease with increasing leaf age due to declines in foliar nitrogen (Grassi & Magnani 2005) and mesophyll conductance to CO_2 (Niinemets *et al.* 2005). Most leaf production of *A. karroo* occurs in a single flush at the start of the wet season. In October 2005, the unburnt plants had not yet produced a new set of leaves whereas the burnt resprouting plants all had young recently expanded leaves. Despite the high A_{max} of the burnt plants in October 2005, the net daily photosynthetic rates of the burnt and unburnt plants were not significantly different (Fig. 5.5a) because the burnt plants also had high dark respiration rates at this time (Table 5.2). Later in the season when the leaf area of coppicing plants matched that of unburnt plants, i.e. from January onwards, there was no systematic difference between the gas exchange measurements of the burnt and unburnt plants.

The mean morning A_{max} readings of *A. karroo* dropped by *ca.* 50% between January (mid wet season) and September (late dry season) (Fig. 5.2). This drop is similar to, but less than, the drop (60-75%) reported for *Erythrophleum chlorostachys* and *Xanthostemon paradoxus* (Eamus *et al.* 1999) and *Eucalyptus tetrodonta* saplings (Prior *et al.* 1997). These tree species are indigenous to north Australian savannas, which also have wet summers and dry mild winters, and like *A. karroo* lose most of their leaves in the winter, at least as saplings. Declines in A_{max} between the wet and the dry season are likely a response to increased stomatal control and declining foliar N and chlorophyll levels in the ageing leaves (Prior *et al.* 1997; Franco 1998; Eamus *et al.* 1999; Montagu & Woo 1999). Between May and August, coincident with the drop in A_{max}, stomatal conductance (G_s) decreased (Fig. 5.2). However, the C_i:C_a ratio increased slightly, over the same period, which indicates additional non-stomatal limitations to photosynthesis.

During the first 4 to 5 months after topkill, leaf biomass of the resprouting plants recovered faster than stem biomass (Fig. 5.3), and by January 2006, the leaf area of the burnt plants matched that of unburnt plants (Fig. 5.4). Thus, between January and May when photosynthetic rates were high (Fig. 5.2 & Fig. 5.5), the burnt plants would have enjoyed a leaf area that matched that of similar sized unburnt plants. However, despite the high carbon gains that occurred between January and May, most of the shoot growth occurred before January (Fig. 5.9b; and see Chapter 3, Fig. 3.5). Franco (1998) reported that assimilation rates of *Roupla montana*, an evergreen cerrado species, stayed fairly high late into the dry season after stem growth had slowed and suggested that this represented an allocation switch from growth to storage. Prior *et al.* (2004) reported that, in Australian Dry Monsoon Forest, Open Forest and Woodland, stem growth occurs almost exclusively in the wet season despite positive carbon assimilation throughout year. They hypothesized that photosynthates from later in the season were preferentially allocated to below-ground organs to facilitate tree recovery in the case of fire. An alternative, although similar, explanation could be that these photosynthates are used to replenish reserves that were used during spring growth and that the cyclical allocation to aboveground sinks and then belowground sinks is part of the plants normal phenological cycle (Mooney & Chu 1974; Seghieri *et al.* 1995). Plants growing in strongly seasonal climates typically deplete starch reserves during the start of the growing season but are able to replenish these reserves later in the season when conditions for growth deteriorate but still support photosynthesis (Cruz & Moreno 2001; Latt *et al.* 2001).

Although topkill did significantly reduce the total assimilation capacity of the burnt plants (Fig. 5.8), assimilated carbon still far exceeded what was potentially available from root reserves (Fig. 5.10). However, during the initial rapid recovery of canopy, i.e. between September 2005 and January 2006, the growth rate of the new shoots exceeded what was available from concurrent photosynthesis (Fig. 5.9a). In a parallel study of *A. karroo* saplings that were also burnt in August 2005, the starch concentration in the roots of the plants declined by *ca.* 75% between September 2005 and February 2006, as the plants resprouted, but recovered to pre-fire levels by August 2006 (Chapter 3, Fig. 3.2). Thus it seems that root starch reserves were mobilized to supplement the rapid recovery of photosynthetic tissue which then became the primary source of carbon for the rest of the year. Danckwerts (1993) reported that within a few days after defoliation of the grass *Themeda triandra,* the contribution of current photosynthesis to growth exceeded the maximum possible contribution from reserves. However, the grasses with high carbon reserves developed more photosynthetic tissue in this short period than grasses with low carbon reserves, and thus their growth rate remained higher than the grasses with low carbon reserves even after photosynthesis became the major source of carbon. In this study, shoot carbon growth exceeded assimilated carbon for approximately 3 months after topkill. During this time the plants would have been at least partially reliant on root reserves. The difference in the dependence of woody species on reserves compared to herbaceous species (Danckwerts 1993; Richards & Caldwell 1985), i.e. for weeks rather than for days, could be because resprouting woody plants have a proportionally high initial investment in non-photosynthetic tissues. Unlike grasses, woody plants need to invest simultaneously in stem tissue to support their leaf tissue.

The rapid recovery of leaf area, within the first half of the growing season, ensured that the plants had sufficient time to replenish spent reserves while conditions were still favourable for photosynthesis. Landhäusser & Lieffers (2002) reported that if *Populus tremuloides* saplings were topkilled when their root starch concentrations were high, their leaf area and root starch concentrations recovered to pre-treatment values within one year. However, if the saplings were topkilled when root reserves were low, i.e. in the spring, their leaf area recovered to only a third of pre-treatment values after a year and root starch concentrations remained depleted. In this study, topkill occurred just before natural period of canopy expansion and when root carbon reserves are at a maximum (Chapter 3, Fig. 3.2). If fire had been earlier in the dry season, canopy growth might have been retarded for longer and below-optimal conditions for photosynthesis might have lead to longer period of reliance on root reserves. If the fire had been later, the starch reserves would have been partially depleted because of normal canopy expansion and there would have been limited time to rebuild the canopy before the normal period of leaf senescence and deteriorating conditions for photosynthesis commenced.

The importance of concurrent assimilation for recovery has implications for how post-fire limitations on the photosynthetic capacity of a resprouting plant can affect both regrowth rates and the replenishment of used reserves. A severe drought or severe browsing post-fire could prevent canopy development and fire, or any severe disturbance, late in the wet season could inhibit the replenishment of root reserves. According to the sensitivity analysis, the assimilation capacity of the resprouting plants would be especially sensitive to disturbances late in the growing season, i.e. between January and April.

This study only considered shoot carbon that was incorporated into shoot biomass, although in reality the carbon costs of the resprouting plant would have also included stem and root respiration. If these respiration costs had been included in the photosynthesis model the carbon costs of growth and maintenance would have exceeded assimilated carbon for even longer than already predicted. However, as the model predicted that carbon gain continued to increase rapidly after January by which time carbon allocation to shoot biomass had already plateaued it seems unlikely that respiration costs would have exceeded that available from concurrent photosynthesis. Furthermore, one of the main aims of the model was to compare root storage and concurrent photosynthesis as sources of carbohydrates, and presumably respiration costs for growth and maintenance would have been the same regardless of the source of carbohydrates.

Conclusion

In this study, the rapid renewal of the canopy of burnt plants in the beginning of the wet season equipped the plants to take full advantage of optimal conditions for assimilation for the remainder of the wet season. By the end of the first year after topkill, concurrent photoassimilates far exceeded root reserves as a source of carbon. However, the carbon invested in stem and leaf biomass during the first 3 months of recovery, i.e. during the initial rapid recovery of the canopy, exceeded what was concurrently produced by the new leaves. It seems that root reserves supplemented the initial recovery of leaf area. The canopy then became the major source of carbon and assimilated sufficient carbon during the remainder of the year to replenish spent reserves.

Chapter 6 : The effect of shading and defoliation on aboveground growth and reserve carbohydrates of resprouting *Acacia karroo*

Introduction

The role of carbohydrate reserves in recovery after severe defoliation and or aboveground death is contentious (Chapin *et al.* 1990). Although carbohydrate reserves are diminished during resprouting (Chapter 3, this thesis; Bowen & Pate 1993, Canadell & Lopez-Soria 1998, McPherson & Williams 1998; Miyanishi & Kellman 1986; Sakai & Sakai 1998) and poor resprouting capacity is associated with low levels of carbohydrate reserves (Canham *et al.* 1999; Ericsson *et al.* 1980; Jones & Laude 1960; Kays & Canham 1991; Landhäusser & Lieffers 2002) there is also evidence that other nutrients, e.g. nitrogen, might limit regrowth before carbon does (Cruz & Moreno 2003a; Hoffmann *et al.* 2000; Tromp 1983). Furthermore, the overall contribution of reserve carbohydrates to aboveground recovery of grasses (Danckwerts 1993; Richards & Caldwell) and some fire-adapted shrubs (Wildy & Pate 2002) is small compared to the contribution from new photosynthetic tissue.

In South African savannas, prescribed fires are used to manage the landscape (Bond & Archibald 2003; Govender *et al.* 2006; Trollope 1984). One particular management goal is the prevention of bush encroachment (Bond & Archibald 2003; Trollope 1984). Although fires in savannas often kill the aboveground parts of trees and shrubs, referred to as topkill, mortality rates of trees are low (Glitzenstein *et al.* 1995; Trollope 1984; Williams *et al.* 1999) as the plants resprout from the surviving rootstock (Bond & van Wilgen 1996; Frost & Robertson 1987; Gignoux *et al.* 1997; Trollope 1984). Assuming plants require reserve carbohydrates to resprout, a fire regime that progressively lowers the carbohydrate reserves of the undesirable species could eventually lead to mortality and hence population decline of the particular species (Bowen & Pate 1993; Donart & Cook 1970; Miyanishi & Kellman 1986).

A. karroo is generally considered one of the key species responsible for bush thickening in South African savannas (Hoffman & O'Connor 1999; Hoffman *et al.* 1999; O'Connor 1995; Skowno *et al.* 1999). *A. karroo* is also noted as being particularly fire adapted as it can resprout repeatedly after fire (Trollope 1984), and it is abundant in tall fire-prone grasslands (Bond *et al.* 2001). Barring additional major disturbances between topkill events, such as drought or intense browsing, *A. karroo* can replenish reserves that are mobilized during resprouting within a year (Chapter 3). Thus, *A. karroo* populations seem well adapted to survive even the most frequent fire regime. But what would be the effect of severe shading or defoliation between fires? Both shading and defoliation can reduce reserve carbohydrates of woody plants (Cruz *et al.* 2003b; Teague 1989) and can impede the replenishment of reserve carbohydrates after topkill (Bowen & Pate 1993; Landhäusser & Lieffers 2002). *A. karroo* is vulnerable to browsing, both within nature reserves (Bond & Loffell 2002; Bond *et al.* 2001) and outside of them (Scogings & Macanda 2005). *A. karroo* also experiences shading, particularly as juveniles. *A. karroo* seedlings are recruited in the grass layer and persist within this layer as a seedling bank despite the low irradiance levels (O'Connor 1995). In African savannas, the potential combination of frequent fires and browsing might cause a progressive depletion of carbohydrate reserves that could eventually lead to plant mortality and hence shrub eradication (Trollope *et al.* 1984).

This study tested (1) whether resprouting shoots of *Acacia karroo* Hayne rely on carbohydrate reserves, as opposed to concurrent assimilation of the new shoots; and (2) whether shading or defoliation would prevent the replenishments of mobilized carbohydrate reserves. The contribution of reserve carbohydrates to aboveground growth was estimated by limiting the assimilation capacity of topkilled plants by shading and repeated defoliation of the new shoots. The photosynthesis model developed in Chapter 5 was used to estimate if and when the carbon assimilated by the new shoots could account for the growth of the shoots. The model was used to calculate the daily amount of carbon assimilated by a plant based on empirical measurements of photosynthetic rates, light levels and total leaf area.

Methods

Study site

The fieldwork for this study was conducted in a 1 ha fenced enclosure (S 28.20161°, E 32.04218°, elevation 140m) in the Gunjaneni area on the eastern border of the Hluhluwe-iMfolozi Park (HiP). Hluhluwe–iMfolozi Park is a 900 km^2 fenced nature reserve in KwaZulu–Natal, South Africa. The vegetation of the park is predominantly fine-leaved Acacia savanna (Balfour & Howison 2001). The park has a mean annual rainfall ranging from 990mm in the north-western hills to less than 635 mm in the low lying southern regions (Balfour & Howison 2001). The majority of the rain falls during summer thunderstorms (Balfour & Howison 2001). The mean minimum temperature in the park is 13°C and the mean maximum temperature is 35°C (Balfour & Howison 2001). Between 1956 and 1996 the mean fire return interval for the park was 4 years, and approximately 90% of these fires burnt between June and September (i.e. in the dry season) (Balfour & Howison 2001).

The mean (±SE) rainfall between June 2005 and July 2006, recorded at three weather station all within 5 km of Gunjaneni, was 507 ± 70 mm. At these three sites, 78 ± 2 % of the rain fell between October 2005 and March 2006. Between 1956 and 2005, the mean fire interval at Gunjaneni was 3.8 years. Before the current study, the site of the enclosure was last burnt in 2001. Within the enclosure the soils were dark brown, slightly acidic (pH 5.5), fine textured, and derived from sandstone, and dolerite parent material (Oakleaf & Glenrosa) (HiP Research Centre, Soils Map). The tree component in the enclosure was dominated by *Acacia karroo* adults and juveniles, and *Acacia nilotica* adults. The shrub layer was dominated by *Acacia caffra, Dichrostachys cinerea* and *Maytenus heterophylla.* The grass sward was dominated by *Cymbopogon excavatus, Eragrostis curvula, Themeda trianda,* and *Panicum deustum*.

Experimental design

The experiment consisted of two treatments, shading and defoliation, and a control. Each of the groups consisted of 30 *A. karroo* plants that had been burnt and completely topkilled on the 30th August, 2005. Within each group, 10 plants were less than 100 cm tall, 10 plants were between 100 to 200 cm tall, and 10 plants were between 200 to 350 cm tall before the fire. For the shading treatment, individual A-frames, covered with 80% shade cloth, were erected over the plants in mid September 2005 and were only removed to take plant measurements every 3 months. The A-frames were *ca.* 80 cm high and 100 cm wide each way. If a plant outgrew its A-frame, then a second A-frame was attached to the first so that the whole plant was kept in the shade. Under a clear sky, the photosynthetically active radiation underneath the A-frames was reduced from *ca.* 1800 μmol m^{-1} s^{-1} to *ca.* 300 μmol m^{-1} s^{-1}, as verified using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA). For the defoliation treatment, all leaves were removed from the resprouting shoots of the plant each month. The harvested leaves were dried at 70°C for 48 h and weighed.

Aboveground growth and biomass partitioning

The pre-fire size of the plants was determined by measuring the height and the diameter of the dead stem in September 2006. The stem diameter and length of each shoot of each plant resprouting after the August 2005 fire were recorded in October 2006, January 2006, April 2006, and August 2006. For each of the sampling dates, estimates of stem and leaf mass were determined allometrically based on stem volume. Stem volume was calculated as a product of stem basal area and length. To determine the allometric relationships additional shoots were measured, harvested, dried at 70°C for 72 h, and weighed on each of the sampling dates (Chapter 5, Fig. 5.1). At the end of the experiment in August 2006, all aboveground parts of the plants were harvested and weighed in the field. To convert fresh weight (FW) to dry weight (DW), the aboveground parts of 14 of the plants were dried, at 70°C for 72 h, and weighed ($DW=FW\times0.52+3.3$ g, $R^2=0.997$, $n=14$, $p<0.001$).

Starch analysis

For each plant, 30 cm of the taproot, measured from the lowest belowground stem scar, was excavated in August 2006 to determine root starch concentrations. The roots were dried at 70°C for 5 days and then weighed. The roots were milled using a Wiley mill (Arthur H. Thomas Co., Philadelphia, USA) with a 1 mm sieve-plate.

Starch in sub-samples (50 mg) of dried, ground root material was hydrolysed to glucose using the amyloglucosidase–alpha-amylase method described by McCleary *et al.* (1997). The glucose concentration of the resulting solution was determined colorimetrically using the phenol–sulphuric method described by Dubois *et al.* (1956). Prior to the starch determination, soluble sugars were removed from the sub-sample by washing the sub-sample in hot (80°C) 80% ethanol (v/v) for 5 minutes, centrifuging the solution at 760 × *g* for 10 minutes, and discarding the supernatant. This process was repeated three times. Starch was hydrolyzed to glucose by incubating the residue remaining after the sugar extraction for 5 minutes at 100°C with 300 U thermo-stable alpha-amylase (from *Bacillus lichen*, Sigma-Aldrich (Pty) Ltd, Castle Hill, New South Wales, Australia); and then incubating the solution for 1 h at 55°C after adding 20 U purified amyloglucosidase (from *Aspergillus niger*, Sigma-Aldrich). The hydrolysate was centrifuged at 760 × *g* for 10 minutes, and the supernatant was used in the colorimetric procedure. For the colorimetric procedure, 200 µl of 5% phenol was added to 200 µl of sample, and 1 ml of concentrated sulphuric acid was added to the mixture. The colour was allowed to develop for at least 15 minutes before the absorbance was read at 490 nm with a microplate spectrophotometer (PowerWave XS, BioTek, Winooski, Vermont, USA).

Modelled Photosynthetic assimilation

A model was developed to estimate the amount of carbon assimilated by resprouting plants, and the amount of carbon invested in aboveground biomass, for *ca.* 1 year after topkill. For a detailed description of the model refer to Chapter 5. In the model, the daily amount of carbon assimilated by a plant was calculated based on empirical measurements of light saturated photosynthetic rates, light response curves, photosynthetically active radiation (PAR), and the total leaf area of coppicing plants. Because the original model uses light levels recorded in the open, the model had to be modified to accommodate the shaded plants. Light levels under the shade cloth were estimated by a simple rectangular hyperbola (adapted from Charles-Edwards *et al.* 1986 p. 57)

$$I_{sh} = I \times (I_{max} \times Sh)/(I + (I_{max} \times Sh)) \qquad \text{Eqn 1}$$

where I_{sh} is the light (PAR) experienced under the shade cloth, I is the actual light recorded in the open, I_{max} is the maximum light level recorded in the field (2300 µmol

m^{-2} s^{-1}), and *Sh* is the proportion of light transmitted through the shade cloth under maximum light (20%). The model was also modified so that, for the defoliated plants, the actual mass of leaves picked off the plants was used in the model, rather than estimates of leaf mass based on shoot measurements.

Daily values of leaf and shoot biomasses were estimated by interpolating the leaf and shoot biomass data recorded on the sampling dates. Carbon investment in leaf biomass was calculated as a product of the leaf biomass and leaf carbon concentration. Carbon investment in stem biomass was calculated in a similar manner. Carbon investment in shoot biomass was the sum of carbon investment in leaf and shoot biomass.

Results

Shoot growth and biomass partitioning

In August 2006, *ca.* 1 year after the fire in 2005, the aboveground biomass of the control plants was strongly correlated (R^2 = 0.81, n =27, $p<0.001$) to the pre-fire height of the plants (Fig. 6.1a). The relationship between pre-fire height and new aboveground biomass shoot mass was weakened by shading (R^2 = 0.43, n=26, $p<0.001$) (Fig. 6.1b) and defoliation (R^2 = 0.46, n=25, $p<0.001$) (Fig. 6.1c) but remained significant. All data were log transformed prior to performing the statistical analyses. The slope of the regression equations indicated that the effect of shading and defoliation on new growth was more severe for larger plants compared to smaller plants, and that the effect of shading was stronger than that of defoliation. After controlling for variation caused by pre-fire size, both shading and defoliation limited aboveground growth compared to the control plants (separate slopes ANCOVA, $F_{2,72}$=6.5, p=0.002).

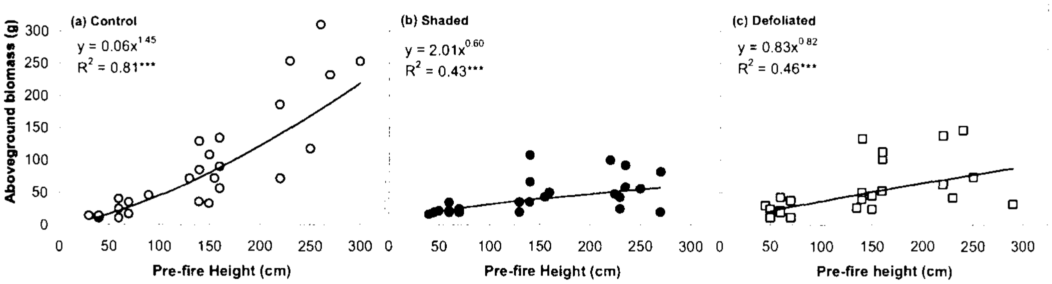

Fig. 6.1. The regression equations and correlation coefficients for aboveground biomass, *ca.* 1 year after the fire, as functions of pre-fire height for the (a) control, (b) shaded, and (c) defoliated plants. All the relationships were positive and significant (*** $p<0.001$), although weaker for the shaded and defoliated plants compared to the control plants.

Shoot elongation slowed considerably after February 2006 for all the plants (Fig. 6.2a–c). Between January and August 2006, there was no significant change in the length of the largest shoots of the control plants (matched pairs t-test, $t_{26}=1.8$, $p=0.08$), the shaded plants ($t_{29}=0.6$, $p=0.6$), or the defoliated plants ($t_{26}=0.3$, $p=0.8$). The shading and defoliation treatments did not significantly inhibit the shoot elongation of the largest shoots of the plants, as there was no significant difference between the groups in January 2006 (ANCOVA, $F_{2,80}=0.3$, $p=0.7$), after the initial growth spurt, or at the end of the experiment in August 2006 (ANCOVA, $F_{2,80}=1.2$, $p=0.3$). Pre-fire height was used as the covariate for the ANCOVA analyses. Shoot thickening stopped after February 2006 for the shaded and defoliated plants, but continued for the control plants until May 2006 (Fig. 6.2d–f). Between January and August 2006, there was no significant change in the basal diameter of the largest shoots of either the shaded plants (matched pairs t-test, $t_{28}=1.1$, $p=0.3$) or the defoliated plants ($t_{26}=1.5$, $p=0.14$), however there was a significant increase in the control plants ($t_{26}=5.5$, $p<0.001$). In January 2006, the mean diameter of the largest shoots of the control plants was significantly larger than that of the shaded plants but not that of the defoliated plants (ANCOVA, $F_{2,80}=8.1$, $p=0.001$). By August 2006, the mean diameter of the largest shoots of the control plants was significantly greater than both that of the shaded and that of the defoliated plants (ANCOVA, $F_{2,80}=14.7$, $p<0.001$). As before, pre-fire height was used as a covariate for the ANCOVA analyses.

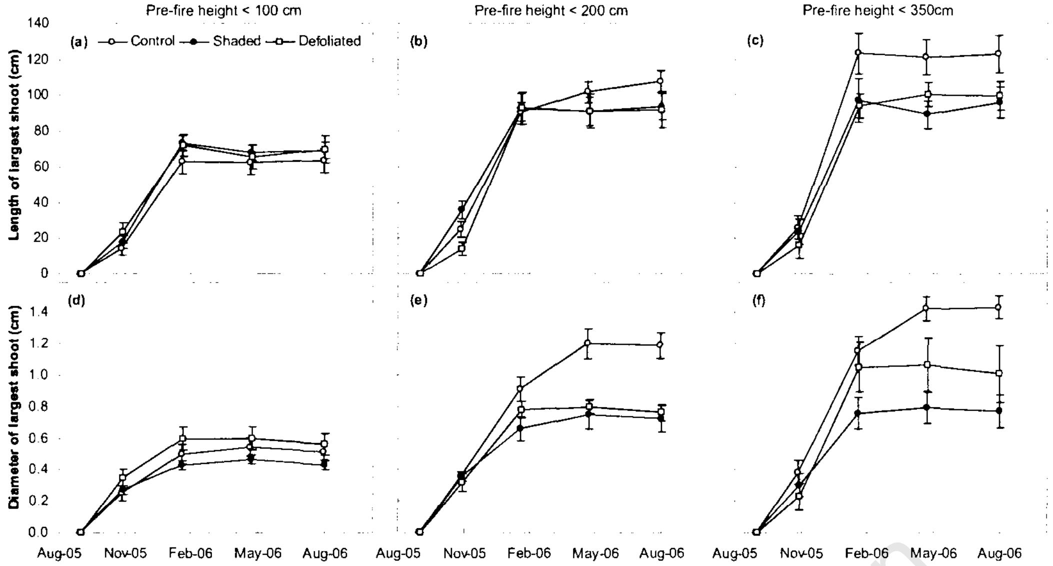

Fig. 6.2. The mean (±SE) length and basal diameter of the largest shoot of the control, shaded and defoliated plants that were topkilled in August 2005. The mean (±SE) values are shown for plants that were less than 100 cm tall (a, d), between 100 to 200 cm tall (b, e), and between 200 to 350 cm tall (c, f) before the fire.

The stem biomass of the control plants continued to increase throughout the dry season (Fig. 6.3), probably because of the continued shoot thickening. The stem biomass of the shaded and defoliated plants also increased during the dry season but at a slower rate than that of the control plants. By August 2006, the mean stem biomass of the largest control plants was more than double that of the largest defoliated and shaded plants (Fig. 6.3c). No new leaf biomass was added to the control and shaded plants after February 2005. However, the defoliated plants continued to develop new leaves throughout the year as compensatory growth was stimulated after defoliation even in the dry winter months (Fig. 6.3d–f).

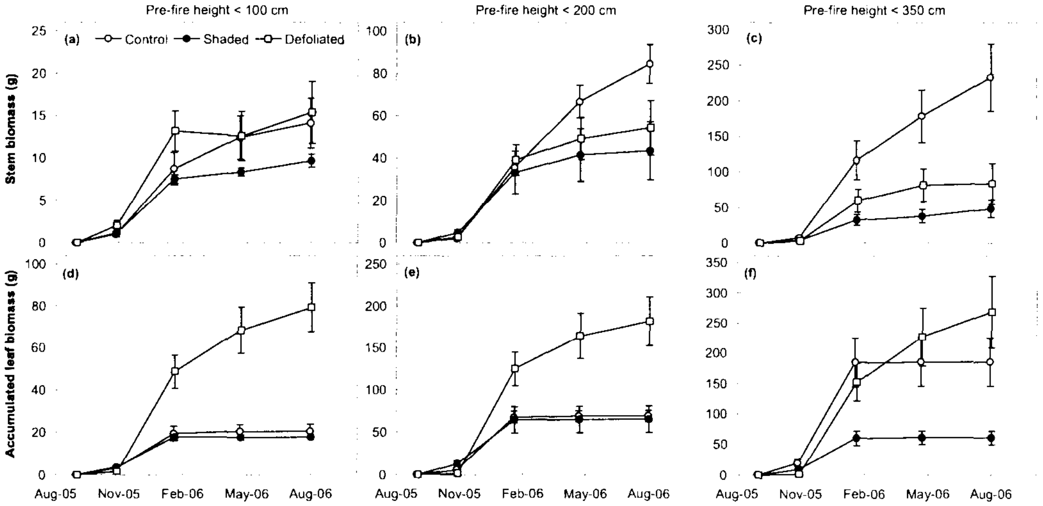

Fig. 6.3. The mean (±SE) stem biomass and accumulated leaf biomass of the control, shaded and defoliated plants that were topkilled in August 2005. The mean (±SE) values are shown for plants that were less than 100 cm tall (a, d), between 100 to 200 cm tall (b, e), and between 200 to 350 cm tall (c, f) before the fire.

For the control and shaded plants, between 70 to 80 percent of new aboveground growth was accounted for by leaf biomass (Fig. 6.4). After January, less than 5% of aboveground growth of the control and shaded plants was due to foliage development. Contrary to this, foliage recovery of the defoliated plants accounted for more than 80% of aboveground growth after January and into the late dry season.

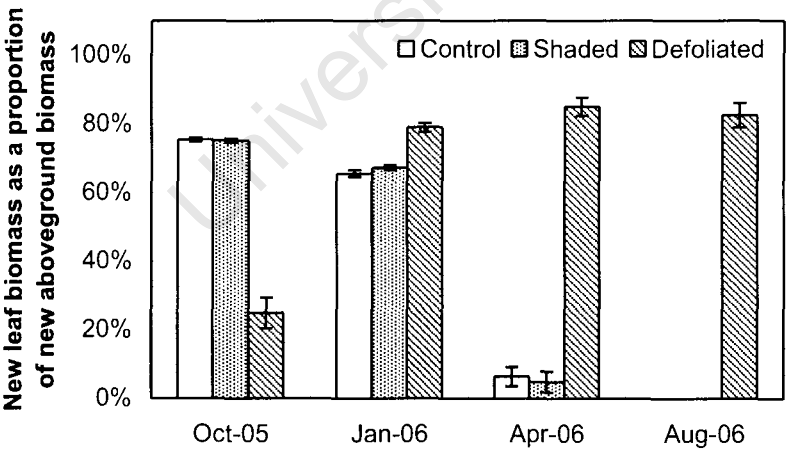

Fig. 6.4. The new leaf biomass accumulated between sampling events as a proportion of the new aboveground biomass.

Root starch concentrations

There was no significant relationship between pre-fire height and root starch concentration of the control plants (R^2=0.06, n=29, p=0.19) so there was no need to control for variation caused by pre-fire size when comparing the root starch concentration of the plants in the different groups. The mean root starch concentration of the plants in the different groups was significantly different (Kruskal–Wallis ANOVA, $H_{2,84}$=53.6, p<0.001) (Fig. 6.5). Whereas shading had the larger effect on aboveground growth, defoliation had the larger effect on root starch accumulation.

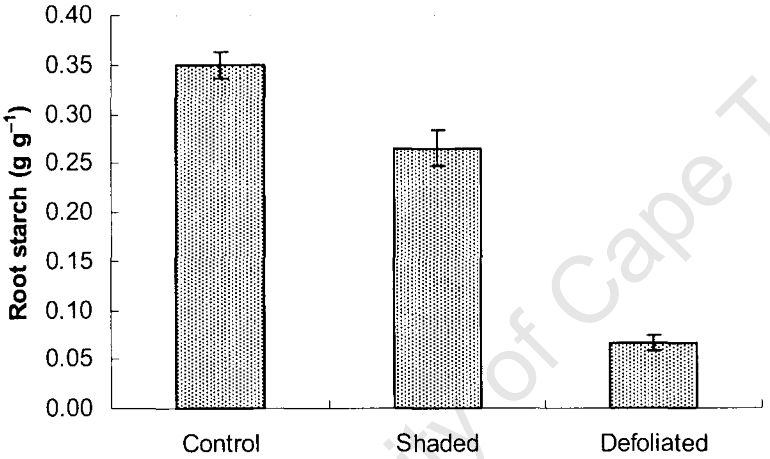

Fig. 6.5. The mean (±SE) root starch concentration of control, shaded, and defoliated plants in August 2006, *ca.* 1 year after the fire (Kruskal–Wallis ANOVA, $H_{2,84}$=53.6, p<0.001). Although both shading and defoliation limited accumulation of root starch compared to the control, defoliation had a stronger effect than shading.

Modelled photosynthetic assimilation

During the year after the plants were burnt, the shoot growth per day was greater than the carbon assimilated per day for 95±3 days for the control plants (Fig. 6.6a), 121±2 days for the shaded plants (Fig. 6.6c), and 292±6 days for the defoliated plants (Fig. 6.6e). Carbon estimated to be in aboveground biomass exceeded the sum of carbon assimilated until the middle of January 2006 for the control plants (Fig. 6.6b) and until the beginning of March for the shaded plants (Fig. 6.6d). Carbon estimated to be in the aboveground biomass of the defoliated plants remained greater than the amount of carbon assimilated by the plants for the duration of the experiment (Fig. 6.6f). By the end of the experiment, only a quarter of the carbon assimilated by the control plants was accounted for by new shoot carbon (Fig. 6.6b). Shoot carbon of shaded plants accounted for approximately half of the carbon assimilated by the shaded plants (Fig. 6.6d), whereas carbon in the shoots of defoliated plants was nearly double that of carbon assimilated (Fig. 6.6f). If the initial root starch concentration of the defoliated plants was 0.35 g g^{-1}, which was the final root starch concentration of the control plants, then the shoot carbon of the defoliated plants would represent 95% ± 3% of the sum of the carbon from mobilized starch and photosynthesis. If the initial pool of starch available to the defoliated plants was calculated using the regression equation shown in Chapter 5, Fig. 5.10c, then the shoot carbon of the defoliated plants represented 99% ± 6% of the sum of carbon from mobilized starch and photosynthesis.

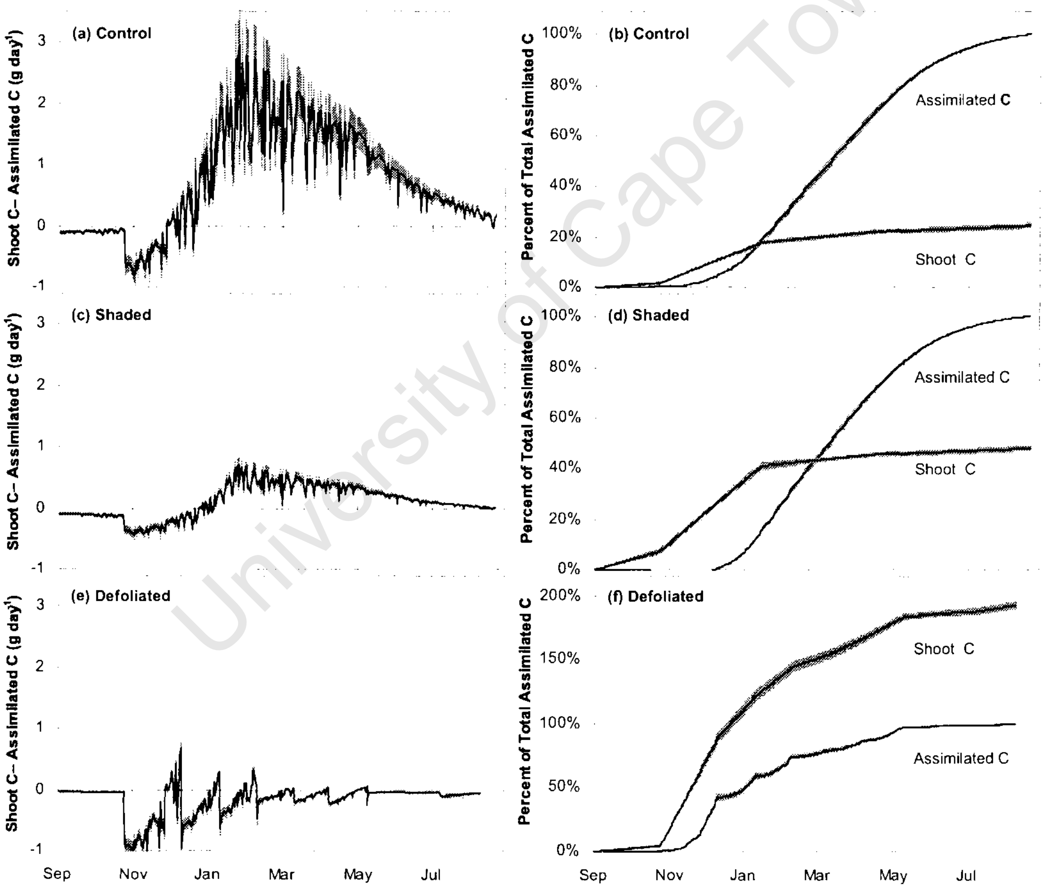

Fig. 6.6. Results of the assimilation model. The mean (±SE) difference between the carbon allocated to shoot biomass and carbon assimilated by the control (a), shaded (c), and defoliated (e) plants on a daily basis. The mean (±SE) cumulative amount of carbon invested in shoot biomass and the mean (±SE) cumulative amount of carbon assimilated by the control (b), shaded (d), and defoliated (f) plants, both expressed as a percentage of the total carbon assimilated by the plants between September 2005 and August 2006.

Plant Mortality

Despite the severe shading and frequent defoliation, mortality rates in all the groups were low. Mortality was 3%, 3%, and 10% for the control, shaded, and defoliated plants, respectively. A chi-squared test showed that the null hypothesis that mortality was evenly spread across the three groups could not be rejected ($\chi^2_{0.05,2}=1.6$, $p > 0.05$).

Discussion

Severe shading and frequent defoliation over a whole year did hamper aboveground growth of coppicing *A. karroo* plants (Fig. 6.1 & Fig. 6.3). However, neither shading nor defoliation significantly limited shoot elongation during the first half of the growing season after the plants were topkilled (Fig. 6.2). After dormant season burning, the root reserves of topkilled *A. karroo* saplings were depleted during the wet season as the canopy was rebuilt (Chapter 3, Fig. 3.2). It appears that during this initial phase of growth, the contribution of mobilized reserve carbohydrates to shoot elongation was sufficient to compensate for reduced photosynthetic capacity caused by shading and defoliation. Kays and Canham (1991) reported a similar result for resprouting *Fraxinus americana* and *Prunus serotina* saplings, i.e. that initial growth was more dependent on reserve carbohydrates than concurrent photosynthesis. An alternative interpretation could be that carbon allocation to shoot growth relative to total carbon gain was small enough that reduced photosynthetic capacity did not affect shoot growth. However, this seems unlikely because according to the assimilation model, carbon invested in shoot biomass by the shaded and defoliated plants exceeded that gained from concurrent assimilation during the first few months after topkill (Fig. 6.6). After the initial phase of rapid shoot elongation that proceeded through the wet season, carbon allocation of the control plants switched from shoot elongation to secondary growth (shoot thickening) (Fig. 6.2). The defoliated and shaded plants did not undergo secondary thickening, hence the final discrepancy in the biomass of the control plants and the shaded and defoliated plants.

Coincident with the cessation of shoot elongation, near the end of the wet season, would be a switch from starch depletion to starch replenishment (Chapter 3). In a parallel study, the root starch concentration of *A. karroo* saplings that were topkilled at the end of the dry season decreased from an annual maximum of *ca.* 0.35 g g^{-1} to *ca.* 0.07 g g^{-1} and then recovered again to maximal values before the end of the dry season (Chapter 3, Fig. 3.2). The root starch reserves declined during the wet season as vegetative growth proceeded rapidly and were replenished over the dry season as vegetative growth slowed down. It this study, the root starch concentrations of the control and shaded plants one year after topkill were well above the minimum recorded for resprouting of *A. karroo* saplings and similar to the recorded annual maximum of *A. karroo* saplings (Fig. 6.5). The final root starch concentration of the defoliated plants was, however, similar to the minimum recorded for resprouting *A. karroo* saplings. Thus, it seems that the defoliated plants were never able to replenish the reserve mobilized to support recovery after topkill and repeated defoliation. Indeed according to the photosynthesis model, both the control and the shaded plants were able to assimilate carbon in excess of the carbon allocated to aboveground biomass by January 2006, 4 months after topkill. The defoliated plants rarely assimilated more carbon than was allocated to aboveground growth. According to the model, all carbon available from assimilation and from mobilized root starch of the defoliated plants would have been required to maintain the shoot growth and particularly the foliage recovery observed in this treatment.

Although defoliation in this study was severe and frequent, less frequent defoliation could still have an effect on reserve carbohydrates; especially if it is concentrated during the start of the dry season when root reserves are normally replenished. After defoliation late in the wet season or early in the dry season, plants would recover their foliage but would be faced with deteriorating conditions for photosynthesis (Chapter 5, Fig. 5.2). Defoliation of temperate tree-crops during the late growing season, when root carbohydrates typically accumulate, prevents the accumulation of root carbohydrate reserves (Loescher *et al.* 1990). The low carbohydrate reserves can then cause death in the following year as spring growth, which relies on reserve carbohydrates, is initiated (Loescher *et al.* 1990). Cutting of American hardwood species towards the end of the growing season, when their root carbohydrate reserves were depleted, prevented the further accumulation of root carbohydrates regardless of the length of the growing season available to the new resprouting shoots (Kays & Canham 1991).

During the initial phase of recovery after topkill, all the plants, regardless of the treatment, invested heavily in rapid leaf area renewal (Fig. 6.4) which was associated with, and perhaps dependent on, rapid shoot elongation (Fig. 6.2). After the initial shoot elongation, the allocation patterns of the plants in the different treatments diverged. The defoliated plants continued to invest heavily in foliage recovery and were not able to invest in starch replenishment or shoot thickening. The control plants allocated carbon both to root starch replenishment and continued shoot growth, through secondary thickening, but not to continued foliage recovery. The shaded plants allocated carbon to root starch replenishment but not to secondary growth or foliage recovery. The rapid leaf area renewal of *A. karroo* after topkill and the preferential allocation to replenishment over continued aboveground growth ensures that even under light limiting conditions mobilized carbohydrates will be replenished, at least partly, within one year. This allocation strategy has important implications for small plants that do not emerge above the grass layer. These small plants suffer from low irradiance levels caused by the dense grass sward (O'Connor 1995) and are especially vulnerable to topkill even by low intensity fires (Trollope 1984). By allocating preferentially to starch recovery after initial shoot recovery the plants ensure that, even under low irradiance levels, they are able to recover from topkill repeatedly. Interestingly, the control plants that were shorter than 100 cm, and presumably would have experienced more shade than the taller individuals, did not show the secondary thickening typical of the taller control plants (Fig. 6.2).

Despite the heavy shading and frequent defoliation, mortality rates of the treated plants were low and not significantly different from that of the control plants. From the severity of defoliation applied in this study it seems that browsing itself might not be able to cause shrub eradication of *A. karroo* if maintained for only a single year post-fire. It also seems that even the most frequent dry season fire regime would not reverse bush thickening caused by *A. karroo* (Chapter 3). Hester *et al.* (2006) report that browsing alone will not reverse bush thickening in the semi-arid savannas of Eastern Cape, South Africa. However, Trollope (1984) reported that in these savanna systems, the combination of continuous goat browsing and annual burning for 5 years led to a 90% reduction in palatable woody species.

If the study reported in this chapter had continued and included a second topkill event, more mortality, particularly of the defoliated plants, might have been recorded. Mortality of resprouting woody plants can occur if reserves are sufficiently depleted before resprouting is initiated (Cruz *et al.* 2003b; Miyanishi & Kellman 1986; Wildy & Pate 2002). However, fire disturbance regimes are rarely frequent enough to cause the necessary depletion of carbohydrate reserves that would lead to mortality of resprouting woody shrubs (Bowen & Pate 1993; Cruz *et al.* 2003b; Miyanishi & Kellman 1986; Wildy & Pate 2002). The combination of frequent fires in savannas and browsing between fires in African savannas could lead to a progressive depletion of root carbohydrate reserves that would lead to reduced resprouting vigour of woody plants and possibly sapling eradication.

Conclusion

The initial rapid shoot elongation that occurred throughout the growing season after topkill of *A. karroo* was not significantly inhibited by shading or defoliation. Thus it seems that during this initial phase, the growth of the new shoots of *A. karroo* is dependent more on mobilised root reserves than on photoassimilates of the new shoots. Frequent defoliation prevented the replenishment of reserves that normally occurs once the canopy has been rebuilt, but shading did not. The combination of frequent fires and heavy browsing between fires has the potential to reverse bush thickening caused by *A. karroo*. What now needs to be determined is what the resprouting response of *A. karroo* would be if it is burnt when root starch concentrations are already depleted; and whether reasonable stocking rates of browsers would maintain the root starch concentration of *A. karroo* at low levels.

Chapter 7 : Contrasting reserve carbohydrate partitioning of fire-tolerant and browser-tolerant savanna species

Introduction

Life-History theory assumes that patterns of resources allocation will be shaped by natural selection to maximise plant fitness (Tilman 1990). Because plants have limited resources, investment in one set of particular traits can carry trade-offs, which can make plants vulnerable to other disturbance agents (Bond & Midgley 2003; Chapin *et al.* 1990; Herms & Mattson 1992). Adaptations and trade-offs will be a function of the most influential selective agent during the evolutionary history of the species or the particular niche that the plant has specialized in (Iwasa & Kubo 1997).

In African savannas, fire and herbivory are the dominant top-down controls of vegetation dynamics (Dublin *et al.* 1990; Langevelde *et al.* 2003; Sankaran *et al.* 2005; Scholes & Walker 1993). However, the severity of fire and herbivory are not homogenous across savannas and often at odds to each other (Archibald *et al.* 2005; Bond & Archibald 2003; Langevelde *et al.* 2003). Browsing and fire might act as different disturbance regimes that select for different allocation patterns. Unlike browsing, fires can cause the complete loss of aboveground tissues. In savannas, aboveground death, or topkill, rarely results in mortality of trees and shrubs but rather induces resprouting (Hoffmann & Solbrig 2003; Frost & Robertson 1987; Trollope 1984). After topkill, root carbohydrate reserves are used for the rapid recovery of the shoot system and the maintenance of the root system until the plant can meet these costs through photosynthesis (Bowen & Pate 1993; Landhäusser & Lieffers 2002; Miyanishi & Kellman 1986). Plants with larger root carbohydrate reserves at the time of topkill are able to resprout more vigorously (Jones & Laude 1960; Kabeya *et al.* 2003; Kays & Canham 1991). Over evolutionary time, fires could select for individuals that actively allocate resources to belowground storage and are thus better able to recover from topkill. Browsing, on the other hand, would not select for belowground storage over aboveground storage as the main stems are rarely lost to browsing.

Midgley and Bond (2001) observed that within the *Acacia* genus, which is ubiquitous across African savannas, there are species that seem particularly fire-tolerant and those that seem particularly browser-tolerant. For example, in the KwaZulu–Natal savannas of South Africa, *Acacia karroo* juveniles are abundant in tall-fire prone grasslands and relatively rare on grazing lawns, whereas the opposite pattern is found for *A. nilotica* juveniles (Bond *et al.* 2001). This landscape pattern seems to result from the contrasting adaptations of *A. karroo* and *A. nilotica; A. karroo* is more likely to be browsed upon than the thornier *A. nilotica,* but *A. karroo* recovers faster after fire than *A. nilotica* (Bond *et al.* 2001).

This study tested the hypotheses that savanna trees and shrubs that are fire-tolerant will preferentially store reserve carbohydrates belowground where they are protected from fire damage, and that this distinction will be more apparent in mesic savannas where fires are more frequent.

Methods

Study site and species

The fieldwork for this study was conducted in Hluhluwe-iMfolozi Park (HiP), KwaZulu-Natal, South Africa (28°00′–28°26′ S; 31°43′–32°09′ E). Hluhluwe–iMfolozi Park is a 900 km^2 fenced nature reserve. The park has a mean annual rainfall ranging from 990mm in the north-western hills to less than 635 mm in the low lying southern regions (Balfour & Howison 2001). The northern half of the park, known as Hluhluwe, is characterised by tall forests and mesic *Acacia* savanna (Whateley & Porter 1983). The southern half of the park, known as iMfolozi, is characterised by broad-leaved thicket and semi-arid *Acacia* savanna (Whateley & Porter 1983). The majority of the rain in the reserve falls during summer thunderstorms (Balfour & Howison 2001). Between 1956 and 1996 the mean fire return interval for the park was 4 years, and approximately 90% of these fires burnt between June and September (i.e. in the dry season) (Balfour & Howison 2001). Fires are generally more frequent in the Hluhluwe Reserve than they are in the iMfolozi reserve (Balfour & Howison 2001) (Fig. 7.1). The park stocks elephant, black rhino, giraffe, kudu, nyala, and impala, which are all either mixed-feeders or browsers (Brooks & MacDonald 1983) that are typical of south-eastern African savannas.

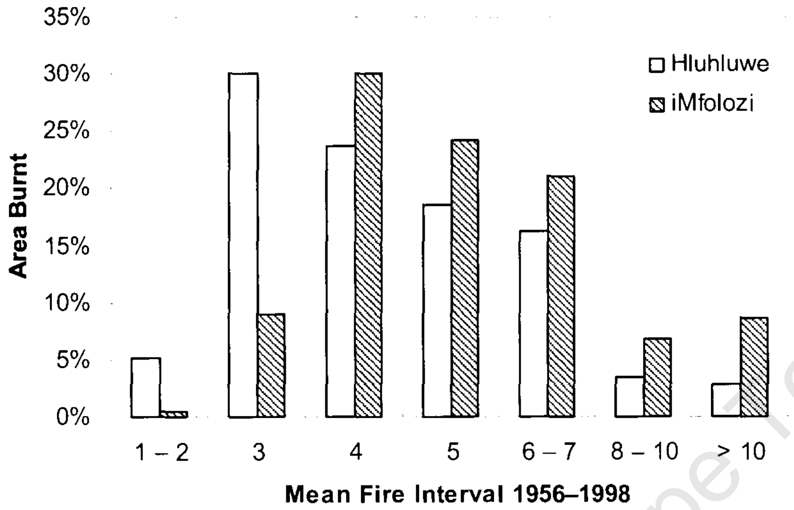

Fig. 7.1: The mean fire return interval for the Hluhluwe Reserve and the iMfolozi Reserve. (Ezemvelo KZN Wildlife, HiP fire records)

Species were identified as being browser-tolerant versus fire-tolerant based on their relative abundance in tall grasslands compared to grazing lawns (Bond *pers. comm.*), on their leaf and spine characteristics, on reported vulnerability to browsing pressure (Bond *et al.* 2001; Bond & Loffell 2001; Du Toit *et al.* 1990), and on their architecture (*sensu* Archibald & Bond 2003). Archibald and Bond (2003) reported that the slender branching pattern of a fire-tolerant form of *A. karroo* facilitated rapid growth out of the flame-zone but left the plant vulnerable to browsing. The highly branched architecture of a browser-tolerant *A. karroo* form provided additional defence against browsing but hampered rapid height growth out of the flame-zone (Archibald & Bond 2003).

Acacia gerrardii, *A. nigrescens* and *Dichrostachys cinerea nyassana,* which are sparsely branched and have relatively small spines, were classified as fire-tolerant species. *A. nilotica*, *A. tortilis* and *D. cinerea africana,* which are densely branched and have relatively large spines, were classified as browser-tolerant species. *A. gerrardii* and *A. nilotica* are generally found in the Hluhluwe Reserve, whereas *A.*

nigrescens and *A. tortilis* are generally found in the iMfolozi Reserve. The two subspecies of *D. cinerea* can be found in both reserves. For a summary of the species sampled see Table 7.1. All the species are fine-leaved, from the Fabaceae family, and indigenous to South African savannas.

Table 7.1: A summary of the species sampled, their life form, whether they were classified as fire- or browser-tolerant, and whether they were harvested in mesic or semi-arid savanna.

Species	**Life Form**	**Guild**	**Savanna**
Acacia gerrardii	Tree	Fire-tolerant	Mesic
Acacia nilotica	Tree	Browser-tolerant	Mesic
Acacia nigrescens	Tree	Fire-tolerant	Semi-arid
Acacia tortilis	Tree	Browser-tolerant	Semi-arid
Dichrostachys cinerea nyassana	Shrub	Fire-tolerant	Mesic
Dichrostachys cinerea africana	Shrub	Browser-tolerant	Semi-arid

Sampling procedure

The stem and taproot of 10 single stemmed individuals between 50 and 150 cm tall of each species were harvested in July 2004. Thirty cm of the stem, measured from the base of the stem, and 30 cm of the root, measured from the lowest belowground stem scar, were harvested. The roots and stems were dried at 70°C for 72 h and milled using a Wiley mill (Arthur H. Thomas Co., Philadelphia, USA) with a 1 mm sieve-plate.

Starch assay

Starch in sub-samples (50 mg) of dried, ground root material were hydrolysed to glucose using the method described by Buysee and Merckx (1993). The glucose concentration of the resulting solution was determined colorimetrically using the phenol–sulphuric method described by Dubois *et al.* (1956). Prior to the starch determination, soluble sugars were removed from the sub-sample by washing the sub-sample in hot (80°C) 80% ethanol (v/v) for 5 minutes, centrifuging the solution at 760 × *g* for 10 minutes, and discarding the supernatant. This process was repeated three times. Starch was hydrolyzed to glucose by incubating the residue, remaining after the sugar extraction, in 10 ml of 3.2% hydrochloric acid for 3 h at 100°C. The hydrolysate was centrifuged at 760 × *g* for 10 minutes, and the supernatant was made up to volume with 80% ethanol. For the colorimetric procedure, 200 µl of 28% phenol (w/w) was added to 200 µl of the sample solution, and 1 ml of concentrated sulphuric acid was added to the mixture. The colour was allowed to develop for at least 15 minutes before the absorbance was read at 490 nm with a spectrophotometer (Spectronic® 20 Genesys^{TM}, Spectronic Instruments, USA).

Data Analysis

Matched pairs t-tests were used to compared the mean starch concentration in the roots and stems of each of the species. The null hypothesis was that there was no difference between the starch concentration in the roots and stems of a plant. A multi-factorial ANOVA was used to compare the root and stem starch concentrations of the fire- vs. browser-tolerant tree species associated with different grass types and the tree species in the different reserves.

Results

The concentration of starch in the roots of the fire adapted tree species was approximately 1.5 times higher than, and significantly greater than, the concentration of starch in their stems (*A. gerrardii* $t_6 = 6.0$, $p = 0.001$; *A. nigrescens* $t_8 = 3.2$, $p = 0.012$) (Fig. 7.2a & b). The concentration of starch in the roots of browser-tolerant tree species was either significantly lower than (*A. nilotica* $t_7 = 2.9$, $p = 0.02$) or not significantly different from (*A. tortilis* $t_8 = 2.0$, $p = 0.08$) the starch concentration in the stems (Fig. 7.2c & d). Neither the fire-tolerant nor the browser-tolerant *D. cinerea* subspecies had starch concentrations in the roots that were significantly different from the starch concentrations in their stems (Fig. 7.2e & f).

The starch concentration in the roots of the fire-tolerant tree species was *ca.* 1.5 times higher than, and significantly greater than, that of the browser-tolerant tree-species ($F_{1,32} = 32.5$, $p < 0.001$). The root starch concentration of the trees from the mesic half of the park were not significantly different from that of trees from the semi-arid half of the park ($F_{1,32} = 0.6$, $p = 0.5$). The starch concentration in the stems of the fire-tolerant trees was not significantly different from that of the browser-tolerant trees;

and the starch concentration in the stems of the trees from the mesic part of the park was not significantly different from that of the trees from the semi-arid part of the park. The root starch concentration in the fire-tolerant *D. cinerea* subspecies was not significantly different from that of the browser-tolerant subspecies ($t_{18} = 0.03$, $p = 0.98$). Overall, the *D. cinerea* subspecies had the lowest concentrations of starch in their roots and stems.

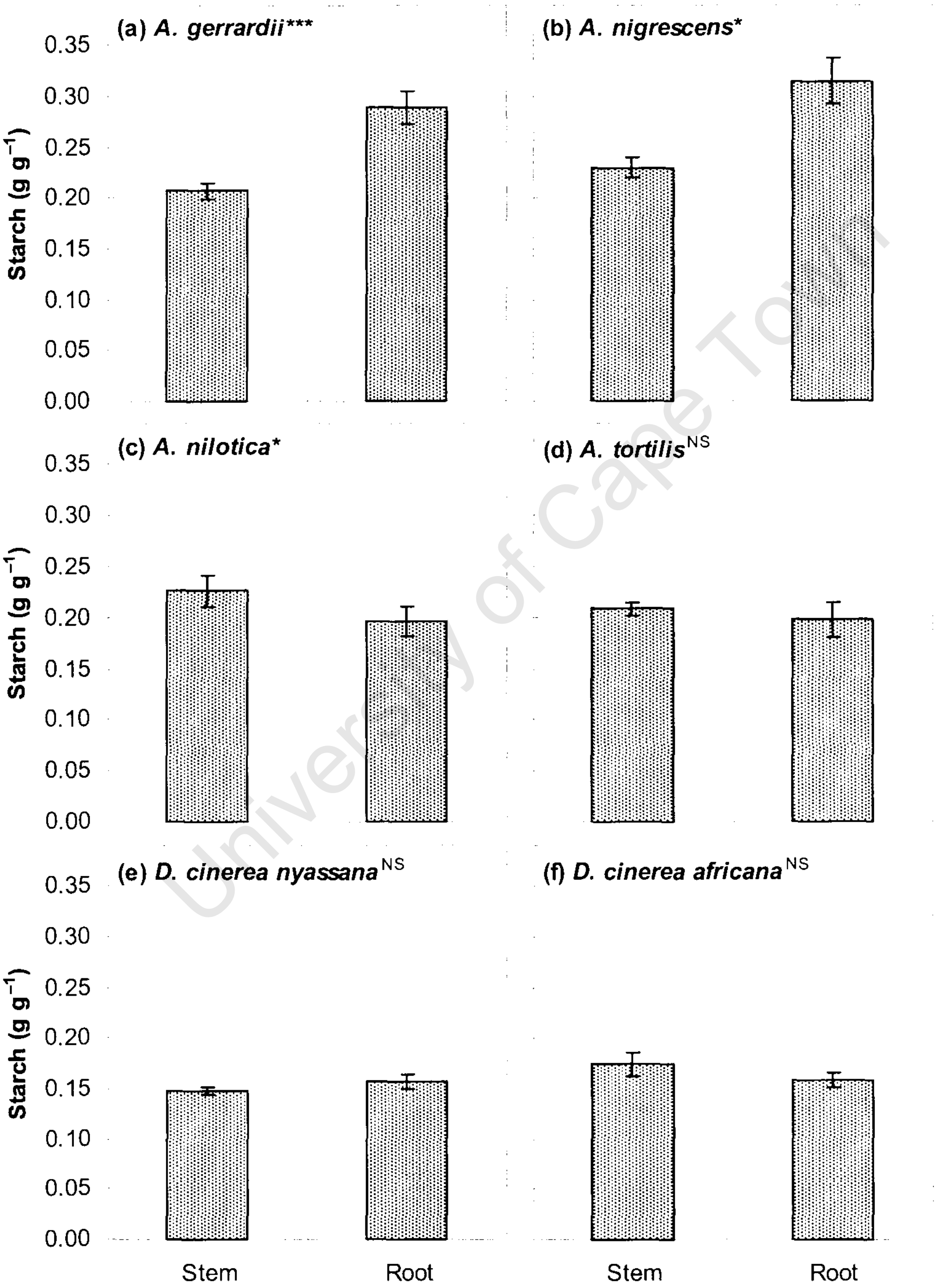

Fig. 7.2: The starch concentration in the stems and roots of the fire-tolerant tree species (a & b), the browser-tolerant tree species (c & d) and the shrub species (e & f). (^{NS} $p > 0.5$, * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.005$)

Discussion

As hypothesized, the trees identified as being fire-tolerant had higher concentrations of starch in their roots than the browser-tolerant tree species. Root starch accumulation has already been identified as a key functional trait of resprouters in Mediterranean-type shrublands (Bell *et al.* 1996; Pate *et al.* 1990; Verdaguer & Ojeda 2002) and in the sclerophyllous shrub species of eastern Australia (Knox & Clarke 2005). The starch concentration in the roots of resprouters in the Epacridaceae family, in south-west Australia, were nearly seven times higher than that of non-sprouting species in the same family (Bell *et al.* 1996). The starch concentration in the roots of the fire-tolerant *Acacia* species were only 1.5 times larger than that of the browser-tolerant *Acacia* species. Although species here have been classified as browser-tolerant or fire-tolerant, even the browser-tolerant species are capable of resprouting after topkill (Bond & Midgley 2000; Luoga *et al.* 2004). Thus it was not unexpected that these species accumulate root starch more than the obligate non-sprouters found in other systems.

The fairly high concentrations of starch in the stems of the tree species could be because they rely on shoot carbohydrates to recover from defoliation. All the species sampled here are capable of sprouting epicormically in response to browser damage (Bond *et al.* 2001; Du Toit *et al.* 1990). Following defoliation, starch concentration in the roots (Chapter 6, Fig. 6.5) and shoots (Teague 1989) of *A. karroo* decreased. Cutting of *Gliricidea sepium* and *Leucaena leucocephala*, which are tree species used in agroforestry, decreased the starch levels in shoots before starch levels in roots were affected (Latt *et al.* 2000). Once stored resources are mobilized they have to be transported to the site of use and this transport incurs additional carbon costs (Chapin *et al.* 1990; Lambers *et al.* 1998). Thus, it should be more efficient for plants to respond to defoliation by mobilizing local reserves, stored in the stems, rather than mobilizing more distant root reserves.

There was no significant difference in the starch concentrations in the roots of the species growing in the mesic half of the park and the semi-arid half of the park. This result was unexpected because it was predicted that the benefit of root storage would be positively correlated to frequency of fire, and hence topkill. Although fires were generally more frequent in the iMfolozi Reserve than in Hluhluwe Reserve over the last half of the last century, almost 60 percent of the iMfolozi Reserve burnt at least once every 5 years (Fig. 7.1). Thus, unless the tree species growing in iMfolozi can grow tall enough to avoid topkill within five years they will need belowground carbohydrate reserves to initiate resprouting. Furthermore, presuming the mesic species grow faster than the semi-arid species, a fire return interval that is relatively long for a mesic species might still be relatively short for a semi-arid species.

Carbohydrate reserves of woody species are not only used during recovery after disturbances; they are also used are also used during normal canopy expansion in the growing season (Kozlowski 1992; Latt *et al.* 2001; Loescher *et al.*1990). The root starch concentration of unburnt *A. karroo* saplings decreased by approximately 50% over the growing season (Chapter 3, Fig. 3.2). Like *A. gerrardii* and *A. nigrescens*, *A. karroo* is fire tolerant, sprouts vigorously, and has high starch concentrations (*ca.* 30%) in its roots at the end of the dry season (Chapter 3). Thus an alternative explanation for the high starch concentrations in the roots and stems of the species sampled could be that these plants rely heavily on reserves to supplement rapid growth at the start of the growing season. However, this explanation does not account for the difference in the concentration of starch in the roots of the fire-tolerant compared to the browser-tolerant trees.

The starch concentration in the stems and roots of both subspecies of *D. cinerea* were substantially lower than that of the other species sampled. Maze (2001) also reported low starch concentrations in the roots of *D. cinerea* compared to other African *Acacias*. Unlike the *Acacia* species, the *D. cinerea* individuals harvested did not have an obvious and swollen taproot. Although *D. cinerea* can resprout basally like the *Acacia* species, it can also send up root suckers, which the *Acacia* species can not do. These root suckers develop on shallow roots distant from the original taproot and can become independent of the mother plant (Wakeling 2004; Ward 1962). Whereas most individuals of *Acacia* species will die if their original rootstock is severely damaged (Maze 2001; Ward 1962), *D. cinerea* individuals can survive such damage by sending up root suckers from undamaged lateral roots and establishing a new root nucleus. *D. cinerea* was also the only species sampled that starts fruiting before it has grown tall enough to escape fire-induced topkill (Schutz 2003). In fact, *D. cinerea* rarely grows tall enough to escape topkill, but completes its whole life-cycle within flame height, unlike the *Acacia* species sampled. Perhaps there is no advantage for *D. cinerea* to concentrate reserves in one particular place (organ) as root suckers could be initiated far from this pool. Furthermore, unlike the fire-tolerant tree species, *D. cinerea* does not need to escape the zone of fire damage before it is able to reproduce, either vegetatively or sexually. Whereas the tree species seem to allocate resources to storage for recovery after browsing or topkill, and to rapid growth out of the zone of potential fire and browsing damage, *D. cinerea* allocates resources to precocious reproduction rather than to storage and growth. Perhaps *D. cinerea* does invest heavily in the survival of this original rootstock as it can send up root suckers that will survive even if the original rootstock dies. On the other hand the *Acacia* species seem to invest heavily in the survival of the original rootstock and recovery and growth of the "mother plant".

Conclusion

The results of this study supported the hypothesis that fire-tolerant trees will preferentially store reserves belowground. The results did not support the hypothesis that fire-tolerant trees in mesic savannas would store more carbohydrate reserves than trees in semi-arid savannas. Perhaps the gradient of fire intensity across the reserve was not large enough for such a pattern to emerge. The only shrub species sampled, *D. cinerea*, had substantially lower concentrations of starch in its roots and stems than the tree species sampled. Unlike the fire-tolerant tree species sampled, *D. cinerea* does not seem to rely on root storage for continued survival within the flame-zone.

Chapter 8 : Synthesis and Conclusion

Surviving the fire-trap

Saplings of savanna trees can get trapped into a cycle of surviving fire-induced topkill by resprouting only to be topkilled again a few years later (Bond & van Wilgen 1996; Gignoux *et al.* 1997). After topkill, reserves are needed to support the recovery of photosynthetic tissue and maintain surviving tissues, until the new leaves can meet these demands (Chapin *et al.* 1990; Kobe 1997). To survive frequent topkill events, plants must either have a large pool of reserves that can support multiple resprouting events or the plant must replenish spent reserves between topkill events (Bellingham & Sparrow 2000; Iwasa & Kubo 1997). I hypothesized that resprouting vigour would depend on belowground reserves of carbohydrates. I was particularly interested in whether frequent topkill could progressively deplete belowground storage of saplings caught in the fire-trap, and eventually cause mortality.

Bush encroachment is becoming an increasing threat to savannas and rangelands across the globe (Asner *et al.* 2003; Fensham *et al.* 2005; Roques *et al.* 2001) and this threat is poised to increase under increasing atmospheric CO_2 concentrations (Bond & Midgley 2000; Hoffmann *et al.* 2000; Polley *et al.* 2002). Prescribed fires are seen as a tool that can prevent and even reverse bush encroachment (Bond & Archibald 2003; Brooks & MacDonald 1983; Trollope 1984). However, fires can either exacerbate or relieve bush encroachment depending on the fire regime, i.e. the intensity, frequency, and timing of the fire (Trollope 1984; Ward 1962). If prescribed fires are to facilitate tree and shrub eradication then presumably a fire regime that increases mortality of the encroaching species is needed (Miyanishi & Kellman 1986).

Storage depletion and replenishment in burnt plants

The root starch reserves of *A. karroo* were depleted by as much as 75% during resprouting (Fig. 3.2). However, these reserves were replenished within the first growing season after topkill. The growth rate of resprouting *A. karroo* saplings in the second year after two consecutive annual topkill events was not significantly different from the growth rate in the first year (Fig. 3.4). In both years, there was rapid shoot elongation during the first few months of the growing season, whereas shoot thickening proceeded more gradually throughout the growing season (Fig. 3.5). For at least 6 weeks after topkill, stored assimilates were mobilized and incorporated into the biomass of coppicing shoots (Fig. 4.2). During this time, photoassimilates from newly matured leaves were transported acropetally to the younger leaves and developing shoot tip, and there was little basipetal transport of new photoassimilates to root structures (Fig. 4.5). The rapid shoot elongation of resprouting plants coincided with priority allocation to leaf tissue (Fig. 5.3) and the rapid recovery of leaf area (Fig. 5.4). According to the photosynthesis model, this initial growth spurt could not be accounted for by concurrent photosynthesis (Fig. 5.9a). However, photosynthetic rates stayed high after shoot growth had slowed (Fig. 5.9b), and over the entire growing season after topkill, the carbon assimilated by the resprouting plants far exceeded mobilized reserves and shoot growth (Fig. 5.10). The large excess of carbon assimilated during the start of the dry season when shoot growth had slowed explains the ability of resprouting *A. karroo* saplings to replenish mobilized reserves within the first growing season after topkill.

The observed pattern of rapid recovery of leaf tissue, supplemented by carbohydrate reserves, which then has the capacity to rapidly replenish spent reserves, provides a mechanistic explanation of the ability of *A. karroo* saplings to survive repeated annual fires (Bond *et al.* 2001; Trollope 1984). For trees that were less than 2.5 m tall, there was a strong correlation between pre-fire height and new shoot production (Fig. 5.3). This implies that once a plant has reached a certain size it can regrow to this size more quickly after topkill. Thus, as the plant ages it resprouts more vigorously between fires, as hypothesized by Bond and van Wilgen (1996) (Fig. 1.1), and it is more likely to grow tall enough to escape topkill between fires. However, if plants are topkilled once they are above a certain size, i.e. greater than 3m, resprouting vigour declines (Fig. 3.4 & Fig. 3.5) (Watson 1980). The decreased resprouting vigour of these large plants, which were tall enough to flower (Fig. 2.12), could be because these plants had started allocating carbohydrates to reproduction over storage, or because the maintenance of their large root structures consumed a large proportion of the root reserves during recovery and less was available for shoot growth.

Storage depletion and replenishment in unburnt plants

The root starch concentration of unburnt plants also declined as the growing season progressed. Depletion of reserves during canopy construction is typical of plants growing in seasonal climates and often greatest when whole plant leaf area is at its maximum (Tissue & Wright 1995; Wyka 1999; Newell *et al.* 2002; Cruz & Moreno 2001). Reserves of deciduous plants such as neotropical forest understory shrubs (Tissue & Wright 1995) and the alpine herb *Oxytropis sericea* (Wyka 1999) are depleted and replenished within the growing season. The reserves of semi-deciduous neotropical forest tree species (Newell *et al.* 2002) and *Erica australis*, an evergreen Mediterranean shrub (Cruz & Moreno 2001), are depleted over the growing season and replenished during the dry season, as is the case for *A. karroo*. The leaf area of unburnt *A. karroo* saplings peaked in the middle of the wet season and declined as the dry season progressed (Fig. 5.4). The belowground starch reserves of unburnt *A. karroo* saplings reached a minimum towards the end of the wet season (February) and then recovered as the dry season progressed (Fig. 3.2).

Can topkill cause mortality?

In this study, topkill occurred late in the dry season when reserve carbohydrates were at a maximum. What remains to be tested is whether *A. karroo* saplings with already low carbohydrate reserves are more likely to suffer mortality after topkill. *Populus tremuloides* (Landhäusser & Lieffers 2002) and *Adenostoma fasciculatum* (Jones & Laude 1960) resprouted poorly if they were topkilled during the growing season when carbohydrate reserves were already low due to spring flushing. In this study, the starch reserves of unburnt plants were lowest late in the wet season. I predict that repeated topkill late in the wet or early dry season is more likely to cause mortality of *A. karroo* than repeated topkill late in the dry season. In a 30-year fire experiment in a loblolly pine plantation, summer burns caused more mortality of understory hardwoods than winter burns (Robbins & Myers 1992). Like *A. karroo,* these hardwood species deplete their carbohydrate reserves during canopy expansion over the summer and are topkilled by fire (Robbins & Myers 1992). In a fire experiment in north Australian savannas, the height growth of *Eucalyptus tetrodonta* juveniles was reduced compared to unburnt plants if the plants were burnt early in the dry season but not if they were burnt late in the dry season (Prior *et al.* 2006). Like *A. karroo*, *E. tetrodonta* juveniles experience fire-induced topkill (Prior *et al.* 2006). *E. tetrodonta* juveniles are also effectively semi-deciduous (Prior *et al.* 1997), which suggests that their root starch reserves would be low at the start of the dry season as is the case with *A. karroo* saplings. One complication with summer burning is that summer fires are typically less intense and cause less topkill than winter fires because the grass sward is still green in summer (Drewa 2003; Govender *et al.* 2006). It is also putatively bad for the grass layer to be burnt while it is actively growing during the growing season (Trollope 1984). Trollope (1984) suggested that if growing season burns are desired then they should be done during mid-summer droughts when the grasses are inactive and dry; these fire will do minimal damage to the grass sward but are intense enough to cause topkill.

Root carbohydrate reserves can also be kept at low levels by defoliation (Fig. 6.5). In the savannas of the Eastern Cape, South Africa, severe browsing by goats for 16 years did not reverse bush encroachment (Hester *et al.* 2006). However, the combination of continuous browsing and annual fires over 5 years caused a 90% reduction in woody cover of palatable species (Trollope 1984). In commercial rangelands the movement of animals can be actively managed. Increased stocking rates of browsers in encroached areas, specifically during the second half of the growing season when reserve carbohydrates are low, could prevent the replenishment of reserves (Teague & Walker 1988) and thus hamper resprouting if the plant were topkilled later in the dry season. As opposed to commercial rangelands, there is not much control over browser movement in conservation areas. However, stocking rates in conservation reserves are actively managed. For example, in Hluhluwe–iMfolozi Park (HiP), black rhino population levels are kept below the carrying capacity of the reserve. The management of the reserve aims to maintain the black rhino population in HiP at a level that ensures optimal population growth so that it can act as a feeder population for other reserves (Sue van Rensburg, Zululand Regional Ecologist, Pers. comm.). Although higher stocking rates of black rhino would potentially result in a lower growth rate of the black rhino population in the reserve, higher stocking rates could potentially aid bush encroachment control in the reserve.

Future research

Alternative Life-history strategies

The life-history strategy described here for *A. karroo* and for *Piliostigma thonningii* by Gignoux *et al.* 1997 is only one of a number of strategies that fire-tolerant woody plants employ. Whereas *A. karroo* and *P. thonningii* often experience complete topkill and resprout basally, there are other tree species, e.g. *Acacia gerrardii* (pers. obs.) and *Crossopteryx febrifuga* (Gignoux *et al.* 1997), that typically experience only partial topkill and are able to resprout from surviving stems and branches. Both *A. karroo* and *A. gerrardii* only mature once they are fairly tall and invulnerable to topkill (pers. obs.) and, thus, are both under pressure to escape the fire-trap. However, *A. gerrardii* seems to allocate resources to thicker stems and thicker bark as a way of escaping the fire-trap, whereas *A. karroo* allocates resources to storage accumulation and rapid growth (c.f. Gignoux *et al.* 1997).

There are also fire-tolerant woody species that mature within the fire-trap and rarely grow tall enough to escape topkill. One such species is *Dichrostachys cinerea* (Schutz 2003). Alongside *A. karroo*, *D. cinerea* is one of the key species responsible for bush encroachment in southern Africa (Hoffman *et al.* 1999; Roques *et al.* 2001). Like *A. karroo, D. cinerea* is fine-leaved and in the Fabaceae family. However, unlike *A. karroo*, *D. cinerea* does not develop a large taproot (pers. obs.), does not accumulate large root starch reserves (Fig. 7.2; Maze 2001) and is able to send up root suckers. These root suckers can survive even if the mother plant dies (Wakeling 2004; Ward 1962). It seems that *A. karroo* juveniles survive within the fire trap, and eventually escape the fire-trap, by maintaining and developing the original rootstock, whereas *D. cinerea* invests less in the original root stock but rather hedges its bets with many smaller root foci that can become independent if the mother plant dies.

The inherent physiological traits that favour the development of one fire resistant life-history strategy over another are not known. Nor is it known which life-history strategies are favoured by different fire regimes. It is predicted that repeated intense fires, that destroy even the most well protected twigs and stems, would favour basal sprouters, and that fires that are very frequent would favour trees that mature within the fire-trap or can reproduce vegetatively (Bellingham & Sparrow 2000; Bond &

Midgley 2001). The generality of these life-history strategies to savanna systems needs to be investigated further.

Evergreen vs. Deciduous starch cycles

In North American Pine Savannas, the annual cycle of starch depletion and replenishment of the evergreen pines differs from that of the deciduous understory hardwood trees and the two groups of plants are favoured by opposing fire regimes (Robbins & Myers 1992). In southern African savannas, there are a number of resprouting evergreen broadleaved woody species, particularly *Maytenus* and *Euclea* species, that contribute to bush encroachment (Skowno *et al.* 1999; Ward 1962; Whateley & Wills 1996). If the root starch phenology of these broadleaved species differs from that of the fine-leaved species, it might be possible to favour one over the other with a particular fire regime.

Root growth and dieback

The effect of topkill on root biomass was not quantified in this study. Root growth of *Populus tremuloides* saplings was negatively affected by topkill if the plants were topkilled in spring or if topkill was followed by defoliation (Landhäusser & Lieffers 2002). I would predict that, after topkill, resources of *A. karroo* are preferentially allocated to shoot growth over root growth and that root die-back would occur if maintenance costs could not be met.

Defence of root storing tissues

When resources are limiting, allocation to one process, e.g. growth, can happen at the expense of other processes, e.g. storage, or defence (Chapin *et al.* 1990; Herms & Mattson 1992; Mooney & Chu 1974). In *A. karroo* defence would not necessarily be limited to leaves only, as the high concentration of starch in the roots suggests that these tissues would also need to be defended against herbivory. Sixty five percent of the roots that were harvested over this study showed some form of damage from wood boring insects. The ethanol extracts of the dry root tissue that preceded the starch assay gave a solution with an orange tinge, which suggests that soluble secondary metabolites where present in the root tissue. Following severe defoliation, such as happens when plants are topkilled, allocation to defence might suffer as photoassimilates are preferentially allocated to growth (Du Toit *et al.* 1990) and root starch replenishment. If secondary metabolites are important for the defence of starch storing tissues and for leaves, plants that are topkilled frequently might become more palatable and thus more vulnerable to both aboveground and belowground herbivory.

Storage of resources other than carbon

In this study, I only investigate the role of carbohydrate reserves during resprouting of *A. karroo*. However, it is possible that other resources that are necessary for growth, e.g. Nitrogen, are also mobilized from stored reserves (Heilmeier *et al.* 1986; Tromp 1983). Stored nitrogen was important for the rapid leaf area development of the biennial monocarp *Arctium tomentosum* during its second year and contributed 20% of the total nitrogen required for the year (Heilmeier *et al.* 1986). In this study, *A. karroo* invested heavily in leaf biomass during the first few months after topkill, which would have had a high nitrogen cost. It is not known whether this nitrogen was newly acquired or reallocated from storage.

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